REMARKS/ARGUMENTS

Reconsideration and withdrawal of the rejections of the present application are respectfully requested in view of the amendments to the claims and remarks presented herewith, which place the application into condition for allowance.

Status of the Claims and Formal Matters

Claims 1, 7-10, 12-19, and 34, and 36-47 are currently pending in this application. Claims 2-5, 20-33, and 48-270 have been withdrawn from further consideration as allegedly being drawn to a non-elected invention. By this paper, Claims 6, 11, and 35 have been cancelled, and Claims 1, 7-10, 12-15, 18, 19, 34, 36, 38-43, 46, and 47 have been amended, without prejudice, and solely in an effort to expedite prosecution pursuant to the U.S. Patent and Trademark Office Business Goals (65 Fed. Reg. 54604 (September 8, 2000)). New Claims 271-276 have been added. Applicants hereby assert the right to reclaim cancelled or withdrawn subject matter in co-pending applications. No new matter has been introduced by these amendments. Support for the amended recitations can be found throughout the specification as originally filed. In particular, support for new Claim 275 and 276 can be found at, *inter alia*, page 11, lines 5-6 and 11-13 of the instant specification as originally filed.

The amendments as presented herein are not made for purposes of patentability within the meaning of §§101, 102, 103, or 112. Rather, these amendments are made for clarity and to round out the scope of protection to which Applicants are entitled.

Rejections under 35 U.S.C. §102(e)

Claims 1, 6, 11, and 34-35 were rejected under 35 U.S.C. §102(e) as allegedly being anticipated by U.S. Patent No. 6,905,669 (hereinafter "DiMartino"). The Office Action contends that DiMartino teaches the treatment of lymphomas comprising SAHA, wherein the dosage is administered orally and at a dosage from 2-100 mg/m^2. Applicants traverse.

DiMartino relates to methods of treating cancer with a DNA methylation inhibitor (such as 5-azacytidine and 5-aza-2'-deoxycytidine) in combination with a histone deacetylase inhibitor,

which can be, for example, depsipeptide, phenylbutyrate, arginine butyrate, and SAHA. However, DiMartino is fatally deficient. First, the dosage from 2-100 mg/m^2 is for continuous intravenous administration of depsipeptide, not oral administration of SAHA. See Column 7, line 17 of DiMartino. Second, DiMartino does not specifically teach or suggest treatment of cutaneous T-cell lymphoma (CTCL) -- notably, DiMartino does not teach any specific dosages of SAHA for CTCL, whether by oral or intravenous administration. In fact, DiMartino is entirely silent as to oral dosages for any histone deacetylase inhibitor, and certainly not SAHA.

To serve as an anticipating reference under §102, the reference must enable that which it is asserted to anticipate. *Elan Pharmaceuticals, Inc. v. Mayo Foundation for Medical Education & Research* 346 F.3d 1051, 68 U.S.P.Q.2d 1373 (Fed. Cir. 2003). Applicants respectfully submit that in view of the lack of disclosure in DiMartino as to specific treatment of CTCL, or to andy oral dosages of SAHA for CTCL treatment, DiMartino cannot anticipate the claimed invention. The rejection should be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 1, 6-19, and 34-47 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Richon et al (U.S. Patent Application No. 2003/0235588; "Richon") in view of Rubartelli et al (Cancer Res. (1995) 55: 675-680; "Rubartelli").

Applicants note that SAHA has been approved by the FDA as a novel, first-in-class therapeutic for the treatment of CTCL. Exhibit 1 is the FDA-approved label for the use of SAHA (Zolinza) in treating CTCL in patients by orally administering SAHA within the claimed dosage range.

Richon is fatally deficient. Richon is based on the discovery that HDAC inhibitors induce expression of a thioredoxin-binding protein-2 which is further associated with a decrease in the level or activity of thioredoxin (TRX). However, although Richon refers to use of HDAC inhibitors in a laundry list of TRX-mediated diseases (see [0017] US 2003/0235588), Richon does not mention treatment of cutaneous T-cell lymphoma (CTCL), as claimed here -- in fact, Richon does not mention CTCL at all.

Rubartelli does not cure the deficiency of Richon. First, Rubartelli does not mention cutaneous T-cell lymphoma at all -- Rubartelli refers to liver cells; specifically the hepatocarcinoma cell line HepG2, and B-cell lymphoma line . Rubartelli in fact concludes that that different cell types respond differently to thioredoxin and that exogenous and endogenous TRX may have different effects (see, Rubartelli, p. 675, Abstract and left column, second and third full paragraphs). There is nothing in Rubartelli that would lead the skilled artisan to conclude that CTCL is mediated by a TRX mechanism; or that SAHA would be effective in treating CTCL by inhibiting TRX. Therefore, Rubaertelli does not provide the nexus between TRX and treating cutaneous T-cell lymphoma.

Second, there is no teaching or suggestion in Rubartelli or Richon of the claimed doses and dosing schedules for oral treatment of patients with CTCL. To the contrary, Rubartelli is silent regarding oral administration of SAHA at a total daily dose of 200-600 mg as recited in independent claims 1 and 34 and the claims that depend therefrom. In fact, the Examiner concedes that Richon and Rubartelli do not disclose instantly claimed specific administration schedules (Office Action , p. 5).

For these reasons, Richon and Rubartelli fail, alone or in combination, to teach or suggest the claimed invention.

Claims 16-19 were rejected under 35 U.S.C. §103(a) as unpatentable over Richon and Rubartelli and further in view of Kelly et al. (Proc. American Society of Clinical Oncology (2001) 20:87a, Abstract No. 344, "Kelly").

As discussed above, Richon and Rubartelli, alone or in combination, are fatally deficient. Kelly fails to cure the defects of Richon and Rubartelli. Kelly teaches two-hour continuous intravenous administration of SAHA in patients, but not oral administration of SAHA in patients suffering from CTCL. There is no teaching in Kelly as to oral dosages of SAHA at all -- and certainly no mention of the oral doses or schedules in independent claims 1 and 34 of SAHA for treatment of CTCL. The objective of Kelly is to define a safe intravenous dosing schedule for SAHA in patients. As demonstrated below (in the discussion of DiMartino), oral dosing of SAHA produces an unexpected increase in half life compared to IV delivery of SAHA. For this

reason, Kelly cannot cure the deficiencies of Richon and Rubartelli. The rejection should be withdrawn.

In view of the foregoing, Applicants respectfully contend that the instantly claimed subject matter is patentable over any combination of Richon, Rubartelli, and Kelly. Reconsideration and withdrawal of the §103(a) rejection over Richon in view of Rubartelli and Kelly are respectfully requested.

Claims 1, 11-19, 34-35, and 40-47 were rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over DiMartino. Applicants traverse.

DiMartino teaches only continuous intravenous administration of depsipeptide, phenylbutyrate, and arginine butyrate. Notably, DiMartino does not specifically teach treatment of CTCL, nor any specific dosages of SAHA for CTCL treatment, whether by oral or intravenous administration. DiMartino is entirely silent as to oral dosages for any histone deacetylase inhibitor, and certainly not SAHA.

One of ordinary skill in the art would recognize that one simply cannot extrapolate the generic dosages disclosed in DiMartino to apply those specifically to SAHA. Dosing for each HDAC inhibitor must be independently established, especially here where the "genus" of HDAC inhibitors disclosed in DiMartino embraces structurally unrelated compounds. The art is replete with evidence showing that SAHA is structurally different from other HDAC inhibitors like depsipeptide, and phenylbutyrate (referred to in DiMartino), and the "effective" dosing of structurally different HDAC inhibitors is markedly different. See, e.g., Figure 2 of DiMartino, which shows that HDAC inhibitors comprise diverse classes of compounds and have widely divergent structures.

Marks, P.A. et al, (2000) J. Natl. Cancer. Inst. 92(15): 1210-1216 ("Marks") (Exhibit 2), and Sandor, V. et al, (2002) Clin. Cancer Res. 8: 718-728 ("Sandor") (Exhibit 3) enclosed herewith, provide evidence that the skilled artisan, would not rely upon the dosages taught by DiMartino for other HDAC inhibitors to determine the claimed dosages for oral administration of SAHA. Sandor demonstrates that depsipeptide, a structurally distinct compound from sodium butyrate and SAHA, has a significantly different potency, and that depsipeptide and butyrate (two

different HDAC inhibitors) have different mechanisms of cytotoxic action (e.g., Pgp modulation).[1/] Depsipeptide upregulates Pgp to cause drug resistance to depsipeptide. In contrast, Applicants note that high Pgp-expressing cells are not resistant to SAHA. For these reasons, the ordinarily skilled artisan would conclude that each structurally distinct HDAC inhibitor is likely to have different dosages that depend not only on the structure of the inhibitor, but that also depend on differences in potency, specific mechanisms of action, and differences in bioavailability. Marks demonstrates that there is a wide range of HDAC-inhibitory concentrations among the different structural classes of HDAC inhibitors (showing that butyrates require millimolar concentrations to achieve inhibition of HDAC activity and multiple effects on other enzyme systems, Trichostatin A inhibits HDAC at nanomolar concentrations, oxamflatin and benzamide inhibit HDAC activity at micromolar concentrations, and apicidin and trapoxin inhibit HDAC at nanomolar concentrations).

It is significant that in the present invention, SAHA is neither structurally nor pharmacologically similar to depsipeptide, and phenylbutyrate. Because of the lack of similarities, the skilled artisan cannot use the intravenous dosages of depsipeptide and phenylbutyrate taught by DiMartino and to determine oral dosing of SAHA as claimed here. At best, DiMartino teaches that DNA methylation inhibitors can be co-administered with depsipeptide, and phenylbutyrate intravenously at the disclosed dosages, but provides no guidance as to the optimum dosages of SAHA or any of these compounds by oral administration. For the foregoing reasons, DiMartino cannot render the claims obvious under §103(a). The rejection in view of DiMartino should be withdrawn.

1/ Sandor, at page 719, col. 1, lines 8-10, expressly states that, "[d]epsipeptide, however, is structurally distinct from other known HDAC inhibitors, such as the trichostatins and trapoxins, and may have other mechanisms of cytotoxic action." Further, at page 725, Sandor teaches that "[u]nlike sodium butyrate, which has also been studied in clinical trials, depsipeptide is active in the nM range, and the induced Pgp is functional and able to transport rhodamine."

CONCLUSION

Favorable action on the merits is respectfully requested. If any discussion of this Amendment would be deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

Dated: May 10, 2007

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HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ZOLINZA safely and effectively. See full prescribing information for ZOLINZA.

ZOLINZA™ (vorinostat) Capsules
Initial U.S. Approval: 20XX

----------INDICATIONS AND USAGE----------
ZOLINZA is a histone deacetylase (HDAC) inhibitor indicated for:
- Treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma (CTCL) who have progressive, persistent or recurrent disease on or following two systemic therapies. (1)

---------- DOSAGE AND ADMINISTRATION----------
- 400 mg orally once daily with food. (2.1)
- If patient is intolerant to therapy, the dose may be reduced to 300 mg orally once daily with food. If necessary, the dose may be further reduced to 300 mg once daily with food for 5 consecutive days each week. (2.2, 5)

---------- DOSAGE FORMS AND STRENGTHS ----------
- Capsules: 100 mg (3)

----------CONTRAINDICATIONS ----------
- None (4)

----------WARNINGS AND PRECAUTIONS----------
- Pulmonary embolism and deep vein thrombosis have been reported. Monitor patient for pertinent signs and symptoms. (5.1)
- Dose-related thrombocytopenia and anemia have occurred and may require dose modification or discontinuation. (2.2, 5.2, 6)
- Gastrointestinal disturbances (e.g., nausea, vomiting and diarrhea) have been reported. Patients may require antiemetics, antidiarrheals and fluid and electrolyte replacement (to prevent dehydration). (5.3, 6, 17.1)
- Hyperglycemia has been observed. Adjustment of diet and/or therapy for increased glucose may be necessary. (5.4, 5.6)
- QTc prolongation has been observed. Monitor electrolytes and ECGs at baseline and periodically during treatment. (5.5, 5.6)
- Monitor blood cell counts and chemistry tests, including electrolytes, glucose and serum creatinine, every 2 weeks during the first 2 months of therapy and monthly thereafter. (5.6)
- Severe thrombocytopenia and gastrointestinal bleeding have been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet count. (5.7, 7.2)
- Fetal harm can occur when administered to a pregnant woman. Women should be apprised of the potential harm to the fetus. (5.8)

---------- ADVERSE REACTIONS----------
- The most common adverse reactions (incidence ≥20%) are diarrhea, fatigue, nausea, thrombocytopenia, anorexia and dysgeusia. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Merck & Co., Inc. at 1-877-888-4231 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------DRUG INTERACTIONS----------
- Coumarin-derivative anticoagulants: Prolongation of prothrombin time and International Normalized Ratio have been observed with concomitant use. Monitor carefully. (7.1)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: X/200X

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ZOLINZA[1] is indicated for the treatment of cutaneous manifestations in patients with cutaneous T-cell lymphoma who have progressive, persistent or recurrent disease on or following two systemic therapies.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Information

The recommended dose is 400 mg orally once daily with food.

Treatment may be continued as long as there is no evidence of progressive disease or unacceptable toxicity.

ZOLINZA capsules should not be opened or crushed *[see How Supplied/Storage and Handling (16)]*.

2.2 Dose Modifications

If a patient is intolerant to therapy, the dose may be reduced to 300 mg orally once daily with food. The dose may be further reduced to 300 mg once daily with food for 5 consecutive days each week, as necessary.

2.3 Dosing in Special Populations

No information is available in patients with renal or hepatic impairment *[see Pharmacokinetics (12.3)]*.

3 DOSAGE FORMS AND STRENGTHS

100 mg white, opaque, hard gelatin capsules with "568" over "100 mg" printed within radial bar in black ink on the capsule body.

4 CONTRAINDICATIONS

None

5 WARNINGS AND PRECAUTIONS

5.1 Thromboembolism

As pulmonary embolism and deep vein thrombosis have been reported as adverse reactions, physicians should be alert to the signs and symptoms of these events, particularly in patients with a prior history of thromboembolic events *[see Adverse Reactions (6)]*.

5.2 Hematologic

Treatment with ZOLINZA can cause dose-related thrombocytopenia and anemia. If platelet counts and/or hemoglobin are reduced during treatment with ZOLINZA, the dose should be modified or therapy discontinued. *[See Dosage and Administration (2.2), Warnings and Precautions (5.6) and Adverse Reactions (6).]*

5.3 Gastrointestinal

Gastrointestinal disturbances, including nausea, vomiting and diarrhea, have been reported *[see Adverse Reactions (6)]* and may require the use of antiemetic and antidiarrheal medications. Fluid and electrolytes should be replaced to prevent dehydration *[see Adverse Reactions (6.1)]*. Pre-existing nausea, vomiting, and diarrhea should be adequately controlled before beginning therapy with ZOLINZA.

5.4 Hyperglycemia

Hyperglycemia has been observed in patients receiving ZOLINZA *[see Adverse Reactions (6.1)]*. Serum glucose should be monitored, especially in diabetic or potentially diabetic patients. Adjustment of diet and/or therapy for increased glucose may be necessary.

5.5 QTc Prolongation

A definitive study of the effect of vorinostat on QTc has not been conducted. Three of 86 CTCL patients exposed to 400 mg once daily had Grade 1 (>450-470 msec) or 2 (>470-500 msec or increase of >60 msec above baseline) clinical adverse events of QTc prolongation. In a retrospective analysis of three Phase 1 and two Phase 2 studies, 116 patients had a baseline and at least one follow-up ECG. Four patients had Grade 2 (>470-500 msec or increase of >60 msec above baseline) and 1 patient had Grade 3 (>500 msec) QTc prolongation. In 49 non-CTCL patients from 3 clinical trials who had complete evaluation of QT interval, 2 had QTc measurements of >500 msec and 1 had a QTc prolongation of >60 msec.

5.6 Monitoring: Laboratory Tests

Careful monitoring of blood cell counts and chemistry tests, including electrolytes, glucose and serum creatinine, should be performed every 2 weeks during the first 2 months of therapy and monthly thereafter. Electrolyte monitoring should include potassium, magnesium and calcium. Baseline and periodic ECGs should be performed during treatment. ZOLINZA should be administered with particular

caution in patients with congenital long QT syndrome, and patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation. Hypokalemia or hypomagnesemia should be corrected prior to administration of ZOLINZA, and consideration should be given to monitoring potassium and magnesium in symptomatic patients (e.g., patients with nausea, vomiting, diarrhea, fluid imbalance or cardiac symptoms). *[See Warnings and Precautions (5.5).]*

5.7 Other Histone Deacetylase (HDAC) Inhibitors

Severe thrombocytopenia and gastrointestinal bleeding have been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet count every 2 weeks during the first 2 months. *[See Drug Interactions (7.2)].*

5.8 Pregnancy

Pregnancy Category D

ZOLINZA can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of ZOLINZA in pregnant women. Results of animal studies indicate that vorinostat crosses the placenta and is found in fetal plasma at levels up to 50% of maternal concentrations. Doses up to 50 and 150 mg/kg/day were tested in rats and rabbits, respectively (~0.5 times the human exposure based on $AUC_{0\text{-}24\ hours}$). Treatment-related developmental effects including decreased mean live fetal weights, incomplete ossifications of the skull, thoracic vertebra, sternebra, and skeletal variations (cervical ribs, supernumerary ribs, vertebral count and sacral arch variations) in rats at the highest dose of vorinostat tested. Reductions in mean live fetal weight and an elevated incidence of incomplete ossification of the metacarpals were seen in rabbits dosed at 150 mg/kg/day. The no observed effect levels (NOELs) for these findings were 15 and 50 mg/kg/day (<0.1 times the human exposure based on AUC) in rats and rabbits, respectively. A dose-related increase in the incidence of malformations of the gall bladder was noted in all drug treatment groups in rabbits versus the concurrent control. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

6 ADVERSE REACTIONS

The most common drug-related adverse reactions can be classified into 4 symptom complexes: gastrointestinal symptoms (diarrhea, nausea, anorexia, weight decrease, vomiting, constipation), constitutional symptoms (fatigue, chills), hematologic abnormalities (thrombocytopenia, anemia), and taste disorders (dysgeusia, dry mouth). The most common serious drug-related adverse reactions were pulmonary embolism and anemia.

6.1 Clinical Trials Experience

The safety of ZOLINZA was evaluated in 107 CTCL patients in two single arm clinical studies in which 86 patients received 400 mg once daily.

The data described below reflect exposure to ZOLINZA 400 mg once daily in the 86 patients for a median number of 97.5 days on therapy (range 2 to 480+ days). Seventeen (19.8%) patients were exposed beyond 24 weeks and 8 (9.3%) patients were exposed beyond 1 year. The population of CTCL patients studied was 37 to 83 years of age, 47.7% female, 52.3% male, and 81.4% white, 16.3% black, and 1.2% Asian or multi-racial.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Common Adverse Reactions

Table 1 summarizes the frequency of CTCL patients with specific adverse events, regardless of causality, using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3.0).

Table 1
Clinical or Laboratory Adverse Events Occurring in CTCL Patients
(Incidence ≥10% of patients)

	ZOLINZA 400 mg once daily (N=86)			
Adverse Events	**All Grades**		**Grades 3-5***	
	n	**%**	**n**	**%**
Fatigue	45	52.3	3	3.5
Diarrhea	45	52.3	0	0.0
Nausea	35	40.7	3	3.5
Dysgeusia	24	27.9	0	0.0
Thrombocytopenia	22	25.6	5	5.8
Anorexia	21	24.4	2	2.3
Weight Decreased	18	20.9	1	1.2
Muscle Spasms	17	19.8	2	2.3
Alopecia	16	18.6	0	0.0
Dry Mouth	14	16.3	0	0.0
Blood Creatinine Increased	14	16.3	0	0.0
Chills	14	16.3	1	1.2
Vomiting	13	15.1	1	1.2
Constipation	13	15.1	0	0.0
Dizziness	13	15.1	1	1.2
Anemia	12	14.0	2	2.3
Decreased Appetite	12	14.0	1	1.2
Peripheral Edema	11	12.8	0	0.0
Headache	10	11.6	0	0.0
Pruritus	10	11.6	1	1.2
Cough	9	10.5	0	0.0
Upper Respiratory Infection	9	10.5	0	0.0
Pyrexia	9	10.5	1	1.2

* No Grade 5 events were reported.

The frequencies of more severe thrombocytopenia, anemia *[see Warnings and Precautions (5.2)]* and fatigue were increased at doses higher than 400 mg once daily of ZOLINZA.

Serious Adverse Reactions

The most common serious adverse events, regardless of causality, in the 86 CTCL patients in two clinical studies were pulmonary embolism reported in 4.7% (4/86) of patients, squamous cell carcinoma reported in 3.5% (3/86) of patients and anemia reported in 2.3% (2/86) of patients. There were single events of cholecystitis, death (of unknown cause), deep vein thrombosis, enterococcal infection, exfoliative dermatitis, gastrointestinal hemorrhage, infection, lobar pneumonia, myocardial infarction, ischemic stroke, pelvi-ureteric obstruction, sepsis, spinal cord injury, streptococcal bacteremia, syncope, T-cell lymphoma, thrombocytopenia and ureteric obstruction.

Discontinuations

Of the CTCL patients who received the 400-mg once daily dose, 9.3% (8/86) of patients discontinued ZOLINZA due to adverse events. These adverse events, regardless of causality, included anemia, angioneurotic edema, asthenia, chest pain, exfoliative dermatitis, death, deep vein thrombosis, ischemic stroke, lethargy, pulmonary embolism, and spinal cord injury.

Dose Modifications

Of the CTCL patients who received the 400-mg once daily dose, 10.5% (9/86) of patients required a dose modification of ZOLINZA due to adverse events. These adverse events included increased serum creatinine, decreased appetite, hypokalemia, leukopenia, nausea, neutropenia, thrombocytopenia and vomiting. The median time to the first adverse event resulting in dose reduction was 42 days (range 17 to 263 days).

Laboratory Abnormalities

Laboratory abnormalities were reported in all of the 86 CTCL patients who received the 400-mg once-daily dose.

Increased serum glucose was reported as a laboratory abnormality in 69% (59/86) of CTCL patients who received the 400-mg once daily dose; only 4 of these abnormalities were severe (Grade 3). Increased serum glucose was reported as an adverse event in 8.1% (7/86) of CTCL patients who received the 400-mg once daily dose. *[See Warnings and Precautions (5.4).]*

Transient increases in serum creatinine were detected in 46.5% (40/86) of CTCL patients who received the 400-mg once daily dose. Of these laboratory abnormalities, 34 were NCI CTCAE Grade 1, 5 were Grade 2, and 1 was Grade 3.

Proteinuria was detected as a laboratory abnormality (51.4%) in 38 of 74 patients tested. The clinical significance of this finding is unknown.

Dehydration

Based on reports of dehydration as a serious drug-related adverse event in clinical trials, patients were instructed to drink at least 2 L/day of fluids for adequate hydration. *[See Warnings and Precautions (5.3, 5.6).]*

Adverse Reactions in Non-CTCL Patients

The frequencies of individual adverse events were substantially higher in the non-CTCL population. Drug-related serious adverse events reported in the non-CTCL population which were not observed in the CTCL population included single events of blurred vision, asthenia, hyponatremia, tumor hemorrhage, Guillain-Barré syndrome, renal failure, urinary retention, cough, hemoptysis, hypertension, and vasculitis.

7 DRUG INTERACTIONS

7.1 Coumarin-Derivative Anticoagulants

Prolongation of prothrombin time (PT) and International Normalized Ratio (INR) were observed in patients receiving ZOLINZA concomitantly with coumarin-derivative anticoagulants. Physicians should carefully monitor PT and INR in patients concurrently administered ZOLINZA and coumarin derivatives.

7.2 Other HDAC Inhibitors

Severe thrombocytopenia and gastrointestinal bleeding have been reported with concomitant use of ZOLINZA and other HDAC inhibitors (e.g., valproic acid). Monitor platelet count every 2 weeks for the first 2 months. *[See Warnings and Precautions (5.7).]*

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D [See Warnings and Precautions (5.8)]

8.3 Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from ZOLINZA, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

The safety and effectiveness of ZOLINZA in pediatric patients have not been established.

8.5 Geriatric Use

Of the total number of patients with CTCL in trials (N=107), 46 percent were 65 years of age and over, while 15 percent were 75 years of age and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

8.6 Use in Patients with Hepatic Impairment

Vorinostat was not evaluated in patients with hepatic impairment. As vorinostat is predominantly eliminated through metabolism, patients with hepatic impairment should be treated with caution. *[See Clinical Pharmacology (12.3).]*

8.7 Use in Patients with Renal Impairment

Vorinostat was not evaluated in patients with renal impairment. However, renal excretion does not play a role in the elimination of vorinostat. Patients with pre-existing renal impairment should be treated with caution. *[See Clinical Pharmacology (12.3).]*

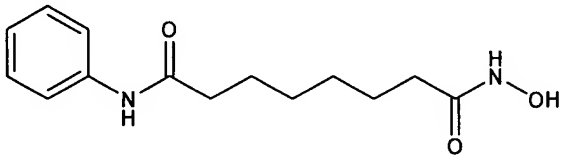
10 OVERDOSAGE

No specific information is available on the treatment of overdosage of ZOLINZA.

In the event of overdose, it is reasonable to employ the usual supportive measures, e.g., remove unabsorbed material from the gastrointestinal tract, employ clinical monitoring, and institute supportive therapy, if required. It is not known if vorinostat is dialyzable.

11 DESCRIPTION

ZOLINZA contains vorinostat, which is described chemically as *N*-hydroxy-*N'*-phenyloctanediamide. The empirical formula is $C_{14}H_{20}N_2O_3$. The molecular weight is 264.32 and the structural formula is:



Vorinostat is a white to light orange powder. It is very slightly soluble in water, slightly soluble in ethanol, isopropanol and acetone, freely soluble in dimethyl sulfoxide and insoluble in methylene chloride. It has no chiral centers and is non-hygroscopic. The differential scanning calorimetry ranged from 161.7 (endotherm) to 163.9°C. The pH of saturated water solutions of vorinostat drug substance was 6.6. The pKa of vorinostat was determined to be 9.2.

Each 100 mg ZOLINZA capsule for oral administration contains 100 mg vorinostat and the following inactive ingredients: microcrystalline cellulose, sodium croscarmellose and magnesium stearate. The capsule shell excipients are titanium dioxide, gelatin and sodium lauryl sulfate.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Vorinostat inhibits the enzymatic activity of histone deacetylases HDAC1, HDAC2 and HDAC3 (Class I) and HDAC6 (Class II) at nanomolar concentrations (IC_{50}<86 nM). These enzymes catalyze the removal of acetyl groups from the lysine residues of proteins, including histones and transcription factors. In some cancer cells, there is an overexpression of HDACs, or an aberrant recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription. Inhibition of HDAC activity allows for the accumulation of acetyl groups on the histone lysine residues resulting in an open chromatin structure and transcriptional activation. *In vitro*, vorinostat causes the accumulation of acetylated histones and induces cell cycle arrest and/or apoptosis of some transformed cells. The mechanism of the antineoplastic effect of vorinostat has not been fully characterized.

12.3 Pharmacokinetics

Absorption

The pharmacokinetics of vorinostat were evaluated in 23 patients with relapsed or refractory advanced cancer. After oral administration of a single 400-mg dose of vorinostat with a high-fat meal, the mean ± standard deviation area under the curve (AUC) and peak serum concentration (C_{max}) and the median (range) time to maximum concentration (T_{max}) were 5.5±1.8 μM•hr, 1.2±0.62 μM and 4 (2-10) hours, respectively.

In the fasted state, oral administration of a single 400-mg dose of vorinostat resulted in a mean AUC and C_{max} and median T_{max} of 4.2±1.9 μM•hr and 1.2±0.35 μM and 1.5 (0.5-10) hours, respectively. Therefore, oral administration of vorinostat with a high-fat meal resulted in an increase (33%) in the extent of absorption and a modest decrease in the rate of absorption (T_{max} delayed 2.5 hours) compared to the fasted state. However, these small effects are not expected to be clinically meaningful. In clinical trials of patients with CTCL, vorinostat was taken with food.

At steady state in the fed-state, oral administration of multiple 400-mg doses of vorinostat resulted in a mean AUC and C_{max} and a median T_{max} of 6.0±2.0 μM•hr, 1.2±0.53 μM and 4 (0.5-14) hours, respectively.

Distribution

Vorinostat is approximately 71% bound to human plasma proteins over the range of concentrations of 0.5 to 50 µg/mL.

Metabolism

The major pathways of vorinostat metabolism involve glucuronidation and hydrolysis followed by β-oxidation. Human serum levels of two metabolites, *O*-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid were measured. Both metabolites are pharmacologically inactive. Compared to vorinostat, the mean steady state serum exposures in humans of the *O*-glucuronide of vorinostat and 4-anilino-4-oxobutanoic acid were 4-fold and 13-fold higher, respectively.

In vitro studies using human liver microsomes indicate negligible biotransformation by cytochromes P450 (CYP).

Excretion

Vorinostat is eliminated predominantly through metabolism with less than 1% of the dose recovered as unchanged drug in urine, indicating that renal excretion does not play a role in the elimination of vorinostat. The mean urinary recovery of two pharmacologically inactive metabolites at steady state was 16±5.8% of vorinostat dose as the *O*-glucuronide of vorinostat, and 36±8.6% of vorinostat dose as 4-anilino-4-oxobutanoic acid. Total urinary recovery of vorinostat and these two metabolites averaged 52±13.3% of vorinostat dose. The mean terminal half-life ($t_{½}$) was ~2.0 hours for both vorinostat and the *O*-glucuronide metabolite, while that of the 4-anilino-4-oxobutanoic acid metabolite was 11 hours.

Special Populations

Based upon an exploratory analysis of limited data, gender, race and age do not appear to have meaningful effects on the pharmacokinetics of vorinostat.

Pediatric

Vorinostat was not evaluated in patients <18 years of age.

Hepatic Insufficiency

Vorinostat was not evaluated in patients with hepatic impairment. *[See Use In Specific Populations (8.6).]*

Renal Insufficiency

Vorinostat was not evaluated in patients with renal impairment. However, renal excretion does not play a role in the elimination of vorinostat. *[See Use In Specific Populations (8.7).]*

Pharmacokinetic effects of vorinostat with other agents

Vorinostat is not an inhibitor of CYP drug metabolizing enzymes in human liver microsomes at steady state C_{max} of the 400 mg dose (C_{max} of 1.2 µM vs IC_{50} of >75 µM). Gene expression studies in human hepatocytes detected some potential for suppression of CYP2C9 and CYP3A4 activities by vorinostat at concentrations higher (≥10 µM) than pharmacologically relevant. Thus, vorinostat is not expected to affect the pharmacokinetics of other agents. As vorinostat is not eliminated via the CYP pathways, it is anticipated that vorinostat will not be subject to drug-drug interactions when co-administered with drugs that are known CYP inhibitors or inducers. However, no formal clinical studies have been conducted to evaluate drug interactions with vorinostat.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been performed with vorinostat.

Vorinostat was mutagenic *in vitro* in the bacterial reverse mutation assays (Ames test), caused chromosomal aberrations *in vitro* in Chinese hamster ovary (CHO) cells and increased the incidence of micro-nucleated erythrocytes when administered to mice (Mouse Micronucleus Assay).

Effects on the female reproductive system were identified in the oral fertility study when females were dosed for 14 days prior to mating through gestational day 7. Doses of 15, 50 and 150 mg/kg/day to rats resulted in approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC. Dose dependent increases in corpora lutea were noted at ≥15 mg/kg/day, which resulted in increased peri-implantation losses were noted at ≥50 mg/kg/day. At 150 mg/kg/day, there were increases in the incidences of dead fetuses and in resorptions.

No effects on reproductive performance were observed in male rats dosed (20, 50, 150 mg/kg/day; approximate exposures of 0.15, 0.36 and 0.70 times the expected clinical exposure based on AUC), for 70 days prior to mating with untreated females. *[See Warnings and Precautions (5.8)]*

14 CLINICAL STUDIES

Cutaneous T-cell Lymphoma

In two open-label clinical studies, patients with refractory CTCL have been evaluated to determine their response rate to oral ZOLINZA. One study was a single-arm clinical study and the other assessed several dosing regimens. In both studies, patients were treated until disease progression or intolerable toxicity.

Study 1

In an open-label, single-arm, multicenter non-randomized study, 74 patients with advanced CTCL were treated with ZOLINZA at a dose of 400 mg once daily. The primary endpoint was response rate to oral ZOLINZA in the treatment of skin disease in patients with advanced CTCL (Stage IIB and higher) who had progressive, persistent, or recurrent disease on or following two systemic therapies. Enrolled patients should have received, been intolerant to or not a candidate for bexarotene. Extent of skin disease was quantitatively assessed by investigators using a modified Severity Weighted Assessment Tool (SWAT). The investigator measured the percentage total body surface area (%TBSA) involvement separately for patches, plaques, and tumors within 12 body regions using the patient's palm as a "ruler". The total %TBSA for each lesion type was multiplied by a severity weighting factor (1=patch, 2=plaque and 4=tumor) and summed to derive the SWAT score. Efficacy was measured as either a Complete Clinical Response (CCR) defined as no evidence of disease, or Partial Response (PR) defined as a ≥50% decrease in SWAT skin assessment score compared to baseline. Both CCR and PR had to be maintained for at least 4 weeks.

Secondary efficacy endpoints included response duration, time to progression, and time to objective response.

The population had been exposed to a median of three prior therapies (range 1 to 12).

Table 2 summarizes the demographic and disease characteristics of the Study 1 population.

Table 2
Baseline Patient Characteristics
(All Patients As Treated)

Characteristics	Vorinostat (N=74)
Age (year)	
Mean (SD)	61.2 (11.3)
Median (Range)	60.0 (39.0, 83.0)
Gender, n (%)	
Male	38 (51.4%)
Female	36 (48.6%)
CTCL stage, n (%)	
IB	11 (14.9%)
IIA	2 (2.7%)
IIB	19 (25.7%)
III	22 (29.7%)
IVA	16 (21.6%)
IVB	4 (5.4%)
Racial Origin, n (%)	
Asian	1 (1.4%)
Black	11 (14.9%)
Other	1 (1.4%)
White	61 (82.4%)
Time from Initial CTCL Diagnosis (year)	
Median (Range)	2.6 (0.0, 27.3)
Clinical Characteristics	
Number of prior systemic treatments, median (range)	3.0 (1.0, 12.0)

The overall objective response rate was 29.7% (22/74, 95% CI [19.7 to 41.5%]) in all patients treated with ZOLINZA. In patients with Stage IIB and higher CTCL, the overall objective response rate was 29.5% (18/61). One patient with Stage IIB CTCL achieved a CCR. Median times to response were 55 and 56 days (range 28 to 171 days), respectively in the overall population and in patients with Stage IIB and higher CTCL. However, in rare cases it took up to 6 months for patients to achieve an objective response to ZOLINZA.

The median response duration was not reached since the majority of responses continued at the time of analysis, but was estimated to exceed 6 months for both the overall population and in patients with Stage IIB and higher CTCL. When end of response was defined as a 50% increase in SWAT score from the nadir, the estimated median response duration was 168 days and the median time to tumor progression was 202 days.

Using a 25% increase in SWAT score from the nadir as criterion for tumor progression, the estimated median time-to-progression was 148 days for the overall population and 169 days in the 61 patients with Stage IIB and higher CTCL.

Response to any previous systemic therapy does not appear to be predictive of response to ZOLINZA.

Study 2

In an open-label, non-randomized study, ZOLINZA was evaluated to determine the response rate for patients with CTCL who were refractory or intolerant to at least one treatment. In this study, 33 patients were assigned to one of 3 cohorts: Cohort 1, 400 mg once daily; Cohort 2, 300 mg twice daily 3 days/week; or Cohort 3, 300 mg twice daily for 14 days followed by a 7-day rest (induction). In Cohort 3, if at least a partial response was not observed then patients were dosed with a maintenance regimen of 200 mg twice daily. The primary efficacy endpoint, objective response, was measured by the 7-point Physician's Global Assessment (PGA) scale. The investigator assessed improvement or worsening in overall disease compared to baseline based on overall clinical impression. Index and non-index cutaneous lesions as well as cutaneous tumors, lymph nodes and all other disease manifestations were also assessed and included in the overall clinical impression. CCR required 100% clearing of all findings, and PR required at least 50% improvement in disease findings.

The median age was 67.0 years (range 26.0 to 82.0). Fifty-five percent of patients were male, and 45% of patients were female. Fifteen percent of patients had Stage IA, IB, or IIA CTCL and 85% of patients had Stage IIB, III, IVA, or IVB CTCL. The median number of prior systemic therapies was 4 (range 0.0 to 11.0).

In all patients treated, the objective response was 24.2% (8/33) in the overall population, 25% (7/28) in patients with Stage IIB or higher disease and 36.4% (4/11) in patients with Sezary syndrome. The overall response rates were 30.8%, 9.1% and 33.3% in Cohort 1, Cohort 2 and Cohort 3, respectively. The 300 mg twice daily regimen had higher toxicity with no additional clinical benefit over the 400 mg once daily regimen. No CCR was observed.

Among the 8 patients who responded to study treatment, the median time to response was 83.5 days (range 25 to 153 days). The median response duration was 106 days (range 66 to 136 days). Median time to progression was 211.5 days (range 94 to 255 days).

15 REFERENCES

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2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. NIH [2002]. 1999 recommendations for the safe handling of cytotoxic drugs. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 92-2621.
4. American Society of Health-System Pharmacists. (2006) ASHP Guidelines on Handling Hazardous Drugs.
5. Polovich, M., White, J. M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

ZOLINZA capsules, 100 mg, are white, opaque hard gelatin capsules with "568" over "100 mg" printed within the radial bar in black ink on the capsule body. They are supplied as follows:

NDC 0006-0568-40.

Each bottle contains 120 capsules.

Storage and Handling

Store at 20-25°C (68-77°F), excursions permitted between 15-30°C (59-86°F). [See USP Controlled Room Temperature.]

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-5] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

ZOLINZA (vorinostat) capsules should not be opened or crushed. Direct contact of the powder in ZOLINZA capsules with the skin or mucous membranes should be avoided. If such contact occurs, wash thoroughly as outlined in the references. Personnel should avoid exposure to crushed and/or broken capsules *[see Nonclinical Toxicology (13.1)]*.

17 PATIENT COUNSELING INFORMATION

[See FDA-Approved Patient Labeling (17.2)]

17.1 Instructions

Patients should be instructed to drink at least 2 L/day of fluid to prevent dehydration and should promptly report excessive vomiting or diarrhea to their physician. Patients should be instructed about the signs of deep vein thrombosis and should consult their physician should any evidence of deep vein thrombosis develop. Patients receiving ZOLINZA should seek immediate medical attention if unusual bleeding occurs. ZOLINZA capsules should not be opened or crushed.

Patients should be instructed to read the patient insert carefully.

Manufactured for:
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

Manufactured by:
Patheon, Inc.
Mississauga, Ontario, Canada L5N 7K9

Printed in USA

XXXXXXX

U.S. Patent Nos. RE 38,506 E, 6,087,367

17.2 FDA-Approved Patient Labeling

[1]Trademark of MERCK & CO., Inc., Whitehouse Station, New Jersey 08889 USA

Patient Information
ZOLINZA™ (zo LINZ ah)
(vorinostat)
Capsules

Read the patient information that comes with ZOLINZA* before you start taking it and each time you get a refill. There may be new information. This leaflet is a summary of the information for patients. Your doctor or pharmacist can give you additional information. This leaflet does not take the place of talking with your doctor about your medical condition or your treatment.

What is ZOLINZA?

ZOLINZA is a prescription medicine used to treat a type of cancer called cutaneous T-cell lymphoma (CTCL) in patients when the CTCL gets worse, does not go away, or comes back after treatment with other medicines.

ZOLINZA has not been studied in children under the age of 18.

What should I tell my doctor before taking ZOLINZA?

Tell your doctor about all of your medical conditions, including if you:

- Have any allergies
- Have had a blood clot in your lung (pulmonary embolus)
- Have had a blood clot in a vein (a blood vessel) anywhere in your body (deep vein thrombosis)
- Have nausea, vomiting, or diarrhea
- Have high blood sugar or diabetes
- Have heart problems
- Are pregnant or plan to become pregnant. ZOLINZA may harm your unborn baby. ZOLINZA has not been studied in pregnant women. If you use ZOLINZA during pregnancy, tell your doctor immediately.
- Are breast-feeding or plan to breast-feed. It is not known if ZOLINZA will pass into your breast milk. Talk to your doctor about the best way to feed your baby while you are taking ZOLINZA.

Tell your doctor about all of the medicines you take, including prescription and non-prescription medicines, vitamins and herbal supplements. Some medicines may affect how ZOLINZA works, or ZOLINZA may affect how your other medicines work. **Especially tell your doctor if you take:**

- **Valproic acid:** a medicine used to treat seizures. Your doctor will decide if you should continue to take valproic acid and may want to test your blood more frequently.
- **COUMADIN®: (warfarin) or any other blood thinner**. Ask your doctor if you are not sure if you are taking a blood thinner. Your doctor may want to test your blood more frequently.

Know the medicines you take. Keep a list of your medicines and show it to your doctor and pharmacist when you get a new medicine.

How should I take ZOLINZA?

- Take ZOLINZA exactly as your doctor tells you to.
- Your doctor will tell you how many ZOLINZA capsules to take and when to take them.
- Swallow each capsule whole. Do not chew or break open the capsule. If you can't swallow ZOLINZA

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*Trademark of Merck & Co., Inc., Whitehouse Station, New Jersey, 08889 USA

capsules whole, tell your doctor. You may need a different medicine.

- Take ZOLINZA with food.
- If ZOLINZA capsules are accidentally opened or crushed, do not touch the capsules or the powder contents of the capsules. If the powder from an open or crushed capsule gets on your skin or in your eyes, wash the contacted area well with plently of plain water. Call your doctor.
- **Drink at least eight 8-ounce glasses of liquids every day while taking ZOLINZA**. Drinking enough fluids may help to decrease the chances of losing too much fluid from your body (dehydration) especially if you are having symptoms such as nausea, vomiting or diarrhea while taking ZOLINZA.
- If you miss a dose, take it as soon as you remember. If you do not remember until it is almost time for your next dose, just skip the missed dose. Just take the next dose at your regular time. Do not take two doses of ZOLINZA at the same time.
- If you take too much ZOLINZA, call your doctor, local emergency room, or poison control center right away.
- Your doctor will check your blood cell counts, blood sugar, and other chemistries every two weeks for the first two months of your treatment with ZOLINZA and then monthly. Your doctor may decide to do other tests to check your health as needed.
- If you have high blood sugar (hyperglycemia) or diabetes, continue to monitor your blood sugar as your doctor tells you to. Your doctor may need to change your diet or medicine to help control your blood sugar while you take ZOLINZA. Be sure to tell your doctor if you are unable to eat or drink normally due to nausea, vomiting or diarrhea.

What are the possible side effects of ZOLINZA?

ZOLINZA may cause **serious side effects**. Tell your doctor right away if you have any of the following symptoms:

- **Blood clots in the legs (deep vein thrombosis)**
  - sudden swelling in a leg
  - pain or tenderness in the leg. The pain may only be felt when standing or walking.
  - increased warmth in the area where the swelling is.
  - skin redness or change in skin color

- **Blood clots that travel to the lungs (pulmonary embolus)**
  - sudden sharp chest pain
  - shortness of breath
  - cough with bloody secretions
  - sweating
  - rapid pulse
  - fainting
  - feeling anxious

- **Dehydration** (loss of too much fluid from the body). This can happen if you are having nausea, vomiting or diarrhea and can not drink fluids well.

- **Low blood cell counts:** Your doctor will periodically do blood tests to check your blood counts.
  - **Low red blood cells.** Low red blood cells may make you feel tired and get tired easily. You may look pale, and feel short of breath.
  - **Low platelets.** Low platelets can cause unusual bleeding or bruising under the skin. Talk to your doctor right away if this happens.

- **High blood sugar** (blood glucose). If you have high blood sugar or diabetes, monitor your blood sugar frequently as directed by your doctor. Tell your doctor right away if your blood sugar is higher than normal.

- **Electrocardiogram abnormality.** An electrocardiogram, or EKG, is a test that records the electrical activity of your heart. Your doctor will check your blood electrolytes and electrocardiogram

periodically.

In addition, the most common side effects with ZOLINZA include:

- **Stomach and intestinal problems,** including diarrhea, nausea, vomiting, loss of appetite, constipation and weight loss
- **Tiredness**
- **Dizziness**
- **Headache**
- **Changes in the way things taste and dry mouth**
- **Muscle aches**
- **Hair loss**
- **Chills**
- **Fever**
- **Upper respiratory infection**
- **Cough**
- **Increase in blood creatinine**
- **Swelling in the foot, ankle and leg**
- **Itching**

Tell your doctor if you have any side effect that bothers you or that does not go away.

These are not all the possible side effects of ZOLINZA. For more information, ask your doctor or pharmacist.

General information about ZOLINZA

Medicines are sometimes prescribed for conditions that are not mentioned in patient information leaflets. Do not use ZOLINZA for a condition for which it was not prescribed. Do not give ZOLINZA to other people, even if they have the same symptoms you have. It may harm them.

Keep ZOLINZA and all medicines out of the reach of children.

This leaflet summarizes the most important information about ZOLINZA. If you would like to know more information, talk to your doctor. You can ask your doctor or pharmacist for information about ZOLINZA that is written for health professionals.

What are the ingredients in ZOLINZA?

Active ingredient: vorinostat

Inactive ingredients: microcrystalline cellulose, sodium croscarmellose and magnesium stearate. The inactive ingredients in the capsule shell are titanium dioxide, gelatin, and sodium lauryl sulfate.

How should I store ZOLINZA?

Store ZOLINZA at room temperature, 68° F to 77° F (20° C to 25°C).

Issued: Month/year

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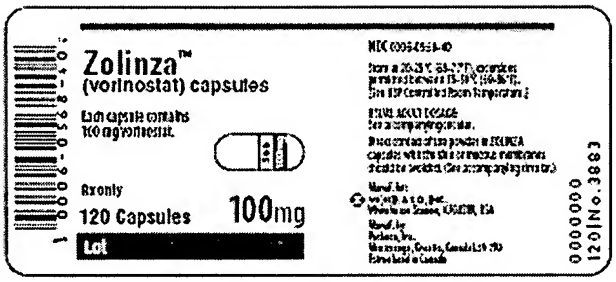
Zolinza™
(vorinostat) capsules
Each capsule contains
100 mg vorinostat.
Rx only
120 Capsules
100mg
Lot
NDC 0006-4561-40
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120 No. 3883

REVIEW

Histone Deacetylase Inhibitors: Inducers of Differentiation or Apoptosis of Transformed Cells

Paul A. Marks, Victoria M. Richon, Richard A. Rifkind

Histone deacetylase (HDAC) inhibitors have been shown to be potent inducers of growth arrest, differentiation, and/or apoptotic cell death of transformed cells *in vitro* and *in vivo*. One class of HDAC inhibitors, hydroxamic acid-based hybrid polar compounds (HPCs), induce differentiation at micromolar or lower concentrations. Studies (x-ray crystallographic) showed that the catalytic site of HDAC has a tubular structure with a zinc atom at its base and that these HDAC inhibitors, such as suberoylanilide hydroxamic acid and trichostatin A, fit into this structure with the hydroxamic moiety of the inhibitor binding to the zinc. HDAC inhibitors cause acetylated histones to accumulate in both tumor and normal tissues, and this accumulation can be used as a marker of the biologic activity of the HDAC inhibitors. Hydroxamic acid-based HPCs act selectively to inhibit tumor cell growth at levels that have little or no toxicity for normal cells. These compounds also act selectively on gene expression, altering the expression of only about 2% of the genes expressed in cultured tumor cells. In general, chromatin fractions enriched in actively transcribed genes are also enriched in highly acetylated core histones, whereas silent genes are associated with nucleosomes with a low level of acetylation. However, HDACs can also acetylate proteins other than histones in nucleosomes. The role that these other targets play in the induction of cell growth arrest, differentiation, and/or apoptotic cell death has not been determined. Our working hypothesis is that inhibition of HDAC activity leads to the modulation of expression of a specific set of genes that, in turn, result in growth arrest, differentiation, and/or apoptotic cell death. The hydroxamic acid-based HPCs are potentially effective agents for cancer therapy and, possibly, cancer chemoprevention. [J Natl Cancer Inst 2000;92:1210–6]

Neoplastic transformation is characterized by inappropriate cell proliferation and/or altered patterns of cell death. However, neoplastic transformation does not necessarily destroy the potential for expression of differentiated characteristics, including cessation of proliferation under appropriate environmental conditions *(1)*. For example, cells infected with temperature-sensitive transforming viruses *(2)* can display either normal or transformed properties, depending on the activity of a temperature-sensitive viral protein. Some malignant cells (e.g., from teratocarcinomas, neuroblastomas, or leukemias) can differentiate along apparently normal pathways when placed in a normal embryonic environment *(3–7)*. In addition, various chemical agents [hybrid polar compounds (HPCs) *(8–10)*, retinoids *(11–15)*, vitamin D_3 *(16)*, and several other agents *(17–19)*] can induce certain transformed cells *in vitro* to express differentiated characteristics and stop proliferating.

Histones are part of the core proteins of nucleosomes. Acetylation and deacetylation of these proteins play a role in the regulation of gene expression *(20)*. There are two classes of enzymes involved in determining the state of acetylation of histones, histone acetyl transferases (HATs) and histone deacetylases (HDACs). There are several reports *(21–24)* that altered HAT or HDAC activity is associated with cancers.

During the last decade, a number of HDAC inhibitors have been identified that induce cultured tumor cells to undergo growth arrest, differentiation, and/or apoptotic cell death *(25–35)*. These agents also inhibit the growth of cancer cells in animal models *(32,35–40)*, and several agents, in particular, hydroxamic acid-based HDAC inhibitors, inhibit tumor growth in animals at doses that are apparently nontoxic and appear to be selective.

This review focuses on studies of HDAC inhibitors, especially on the hydroxamic acid-based HPCs. These compounds represent a class of agents that are potentially effective cancer therapies. (Studies were identified for this review by searching the MEDLINE® database for appropriate papers published in the last 10 years and by a review of bibliographies from articles identified through that search. In addition, we include some of our unpublished data.)

Histone Acetylation and Deacetylation and Gene Expression

Structure of Nucleosomes

Analyses (x-ray and electron crystallographic) show that nucleosomes contain an average of 150 base pairs of DNA wrapped around the nucleosomal core of histones in 1.75 turns of left-handed superhelical DNA *(41–43)*. Five classes of histones have been identified in chromatin: histones H1, H2A, H2B, H3, and H4. Each nucleosome contains two H2As, two H2Bs, two H3s, and two H4s in the core (Fig. 1). Histone H1 occurs in chromatin in about half the amount of the other types of histones and appears to lie on the outer portion of the nucleosome.

Role of Histone Acetylases and Deacetylases

Histones of the nucleosomal core can be acetylated and deacetylated, and the amount of acetylation is controlled by the opposing activities of two types of enzymes, HATs and HDACs. Substrates for these enzymes include ε-amino groups of lysine

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See "Notes" following "References."

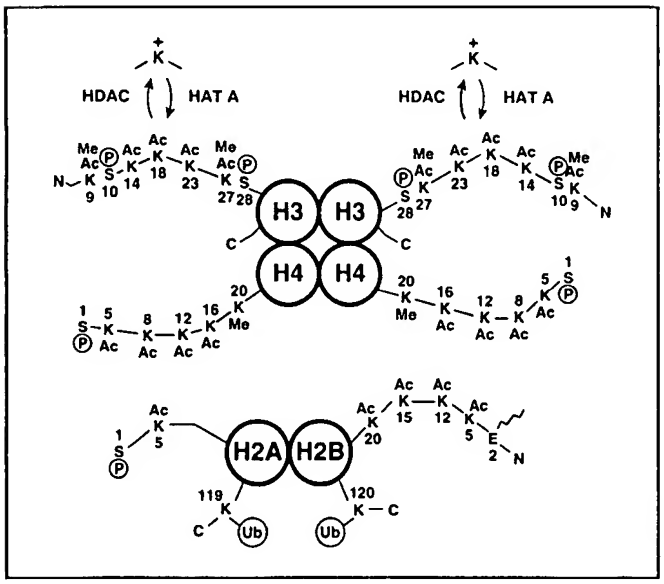


Fig. 1. Histones in nucleosomes. Lysines (K) in the amino (N)-terminal tails of histones H3, H4, H2A, and H2B are potential acetylation/deacetylation sites for histone acetyltransferase (HAT) and histone deacetylase (HDAC). K^+ = positively charged lysine, Ub = ubiquitin, P = phosphate, Ac = acetyl, S = serine, E = glutamic acid, and Me = methyl. [Adapted with permission from Davie *(44).*]

residues located in the amino-terminal tails of the histones. When HDAC removes the acetyl group from histone lysine, it restores a positive charge to the lysine residue condensing the structure of nucleosomes *(44).*

HATs and HDACs

There are at least four groups of proteins with intrinsic HAT activity *(45–50).* The first group contains the GCN5 and P/CAF proteins, which are related to yeast HAT GCN5. The second group contains the closely related cyclic adenosine monophosphate response element-binding protein (CBP) and p300, which act as coactivators for a number of transcription factor complexes. The third group contains the TAF250 protein, part of the basic transcription complex TFIID that binds the TATA box. The fourth group contains the SRC-1 and ACTR proteins that are coactivators for ligand-activated nuclear receptors. In addition, there are probably several other proteins with HAT activity, such as BRCA2, that are part of transcription complexes. HATs play a role in activation of gene expression and may also be involved in gene repression, as suggested by the observation in *Drosophila* that acetylation of the transcription factor T-cell factor by CBP represses transcription *(51).*

Eight HDACs have been described in mammalian cells *(45,52–59).* The yeast RPD3 homologues are HDAC1, HDAC2, HDAC3, and HDAC8; the yeast HDA1 homologues are HDAC4 (also known as HDAC-A), HDAC5 (also known as mHDA1), HDAC6 (also known as mHDAC2), and HDAC7.

Regulation of Transcription

Chromatin fractions enriched in actively transcribed genes are also enriched in highly acetylated core histones *(20,42,45),* whereas silent genes are associated with nucleosomes with a low level of acetylation. Allfrey *(60)* first suggested that histone acetylation was involved in the regulation of transcription. During the past decade, considerable evidence has accumulated to establish the role of acetylation and deacetylation of histones in the regulation of transcription *(20,41–43,45).* The following model describes a role for histone acetylation in regulating gene transcription. Nucleosomes containing highly charged hypoacetylated histones bind tightly to the phosphate backbone of DNA, inhibiting transcription, presumably, because transcription factors, regulatory complexes, and RNA polymerase do not have access to the DNA. Acetylation neutralizes the charge of the histones and generates a more open DNA conformation. Transcription factors and the transcription apparatus then have access to the DNA, and expression of the corresponding genes is promoted (Fig. 2).

In addition to HDACs and HATs, other factors are involved in the regulation of chromatin structure, including methyl-CpG-binding protein *(61–63)* and adenosine triphosphate (ATP)-dependent chromatin-remodeling complexes *(64).* These chromatin-modifying complexes interact with HAT and HDAC complexes to regulate transcriptional activity of genes [for a recent review of chromatin methylation, *see (63);* for reviews of the ATP-dependent chromating remodeling complexes, *see (64,65).*]

HDACs are bound to large protein complexes that regulate gene transcription. Mammalian HDAC1 and HDAC2 are associated with the Sin3 complex that includes NCo-R, SMRT, and several other, as yet, unidentified proteins and appear to repress gene expression by deacetylating core histones. In addition to deacetylation of histones, HDACs may also regulate gene expression by deacetylating transcription factors, such as p53, GATA-1, TFIIE, and TFIIF *(66–68).* HDACs may also participate in cell cycle regulation. The transcription repression mediated by RB binding to the transcription factor E2F involves recruitment of HDAC1 or HDAC2 by RB *(69,70).*

Disruption of HAT and/or HDAC Activity and Development of Cancer

Mutations in the CBP gene, which encodes an HAT, are associated with leukemogenesis and the developmental disorder

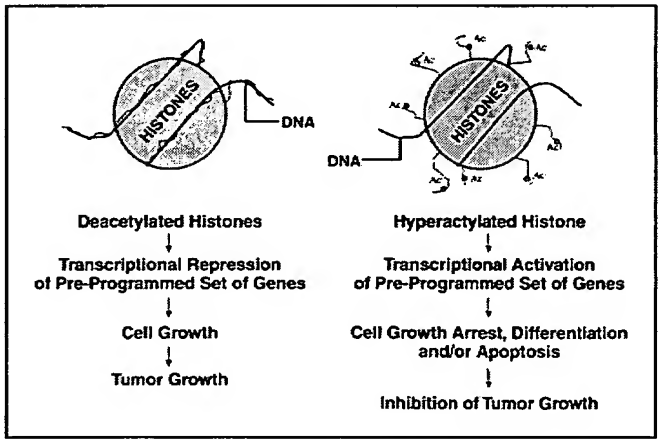


Fig. 2. Proposed mechanism of action of histone deacetylase (HDAC) inhibitors that induce tumor growth arrest, differentiation, and/or apoptotic cell death. With inhibition of HDAC, histones are acetylated (Ac), and the DNA that is tightly wrapped around a deacetylated histone core relaxes. We propose that the accumulation of acetylated histones in nucleosomes leads to expression of specific genes, which, in turn, lead to cell growth arrest, differentiation, and/or apoptotic cell death and, as a consequence, inhibition of tumor growth.

Rubinstein–Taybi syndrome *(71)*. Patients with Rubinstein–Taybi syndrome have a propensity to develop cancer. Microdeletions, translocations, inversions, and various point mutations in the CBP gene have been identified in patients with Rubinstein–Taybi syndrome as well as in patients with some types of colorectal or gastric carcinomas *(21)*. Gene fusions with CBP are associated with several leukemias. In therapy-related acute myeloid and lymphoid leukemias and in myelodysplasia, the CBP gene has been found fused to the MLL gene, and the CBP gene has been found fused in acute myeloid leukemia to the MOZ gene *(72,73)*.

Several leukemogenic transcription factors repress expression of specific genes because of aberrant recruitment of HDACs. This repression of gene expression appears to be an important step in the leukemogenic action of these transcription factors. For example, aberrant recruitment of HDAC activity has been reported in cell lines derived from patients with acute promyelocytic leukemia (APL) *(22–24)*. The oncoprotein encoded by the translocation-generated fusion gene in APL (promyelocytic leukemia [PML]–retinoic acid receptor-α) represses transcription by recruitment of HDAC1. Furthermore, resistance to the differentiating actions of all-*trans*-retinoic acid in a patient with APL was overcome by cotreatment with an inhibitor of HDAC *(74)*. [In a further study *(75)*, four other patients with APL failed to respond.] HDAC-dependent aberrant transcriptional repression is implicated as the main oncogenic mechanism in specific types of myeloid leukemia and lymphoma. For example, in non-Hodgkin's lymphoma, the transcriptional repressor BCL6 is inappropriately overexpressed within the lymphoid compartment, resulting in aberrant transcriptional repression and lymphoid oncogenic transformation *(76)*. Another example is acute myelogenous leukemia of the M2 subtype associated with the t(8;21) chromosomal translocation involving the AML1 and ETO genes *(77)*. The AML1–ETO fusion protein, unlike the AML-1 protein (a transcriptional activator), is a potent dominant transcriptional repressor. In both of these cases, transcriptional repression appears to be mediated by recruitment of HDAC to the transcriptional repressor complex.

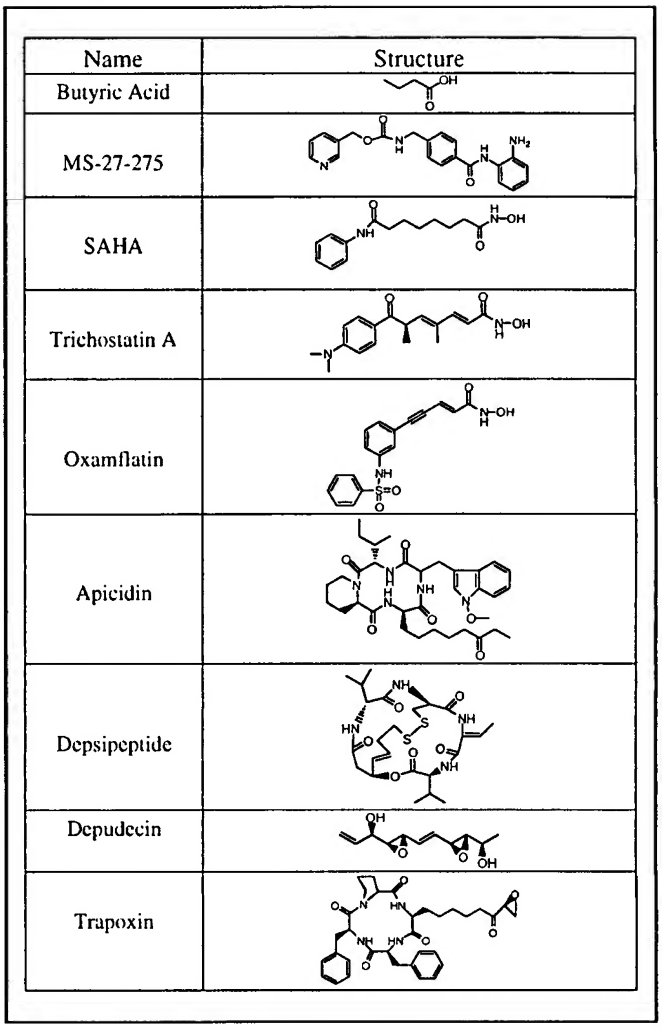
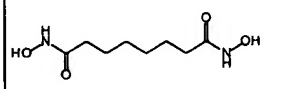
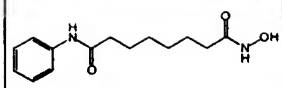
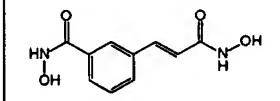
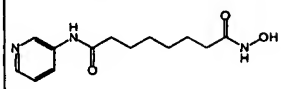


Fig. 3. Histone deacetylase inhibitors (*see* text for references to these inhibitors). SAHA = suberoylanilide hydroxamic acid.

HDAC Inhibitors

Compounds that inhibit HDAC activity are shown in Fig. 3. Several structural classes of HDAC inhibitors have been identified including the following: 1) short-chain fatty acids [e.g., butyrates *(28,31)*]; 2) hydroxamic acids [e.g., trichostatin A (TSA) *(25,26)*, suberoylanilide hydroxamic acid (SAHA) *(34)*, and oxamflatin *(35)*]; 3) cyclic tetrapeptides containing a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety [e.g., trapoxin A) *(27)*]; 4) cyclic peptides not containing the AOE moiety [e.g., FR901228 and apicidin *(33,78)*]; and 5) benzamides [e.g., MS-27-275 *(32)*]. HDAC inhibitors invariably inhibit proliferation of transformed cells in culture, and a subset has been shown to inhibit tumor growth in animal models *(26,32,35–40)*. The butyrates represent the only class that is approved currently for use in the clinic. The butyrates are not ideal agents because of the high concentrations required (millimolar) to achieve inhibition of HDAC activity and multiple effects on other enzyme systems *(28,31)*. TSA, originally developed as an antifungal agent *(25,26,29)*, is a potent inhibitor of HDAC that is active at nanomolar concentrations. The finding that TSA-resistant cell lines have an altered HDAC is evidence that this enzyme is an important target for TSA. Oxamflatin *(35)*, a hydroxamic acid-based compound, and the benzamide MS-27-275 *(32)* inhibit HDAC activity at micromolar concentrations. Apicidin is a fungal metabolite that exhibits potent, broad-spectrum antiprotozoal activity and inhibits HDAC activity at nanomolar concentrations *(78)*. Depsipeptide (FR901228), isolated from *Chromobacterium violaceum (33)*, inhibits HDAC activity at micromolar concentrations. Trapoxin *(27)* and depudecin *(30)* irreversibly bind to HDAC and inhibit its activity at nanomolar and micromolar concentrations, respectively.

In our laboratory, a series of hydroxamic acid-based HPCs have been synthesized that inhibit HDACs at micromolar concentrations or lower *in vitro* and *in vivo (34,36,38,79)* (Fig. 4), and extensive structure–activity studies have been done with these compounds *(34,79)*. The essential characteristics of hydroxamic acid-based HPCs are a polar site, the hydroxamic group, a six-carbon hydrophobic methylene spacer, a second polar site, and a terminal hydrophobic group (Fig. 4). Substitution of the hydroxamic acid with a carboxylic acid or amide oxime group results in inactive compounds. Modification of the hydroxamic acid, such as introduction of a methyl group on an adjacent carbon or *N*-methylation, results in inactive compounds. The benzene ring in the hydrophobic moiety can be

Name	Structure	Opt. Conc.	% Diff.
SBHA		30 μM	90%
SAHA		2.5 μM	68%
CBHA		4.0 μM	73%
Pyroxamide		4.0 μM	51%

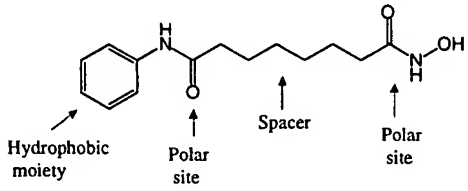


Fig. 4. Hydroxamic acid-based hybrid polar compounds. The optimal concentration to induce murine erythroleukemia cells to differentiate (% Diff) was determined from the percent of differentiated cells [detected as benzidine-stained cells (benzidine binds to the iron-containing heme of hemoglobin); for details of methods, *see (34)*]. SBHA = suberic bishydroxamic acid; SAHA = suberoylanilide hydroxamic acid; CBHA = *m*-carboxy-cinnamic acid bishydroxamic acid.

modified in the meta and para positions without loss of activity; however, in general, larger substituents are associated with loss of activity. The optimal methylene spacer is six methylenes, five- and seven-carbons spacers being less active.

The structure of the catalytic core of HDACs has been determined by x-ray crystallography *(80)*. HDACs share an approximately 390-amino acid region of homology, referred to as the deacetylase core. Residues that form the active site are conserved across all HDACs. The deacetylase core identifies a gene superfamily that includes an HDAC homologue in the hyperthermophilic bacterium *Aquifex aeolicus* (termed "HDLP"), which was used for x-ray crystallography studies. There is a 35.2% base-pair identity between sequences of the catalytic core of the HDLP and of the mammalian HDAC1. HDLP deacetylates histones *in vitro,* its activity is inhibited by TSA and SAHA, but its specific activity is equal to about 7.5% of that of partially purified HDAC1. From x-ray crystallographic analyses of HDLP, an HDLP–TSA complex, and an HDLP–SAHA complex, the active catalytic site in the HDLP was shown to be formed by a tubular pocket, a zinc-binding site, and two asparagine–histidine charge-relay systems (Fig. 5). The hydroxamic acid moieties of TSA and SAHA bind to the zinc in the tubular pocket and the carbon-ring group projects out of the pocket on the surface of the protein.

Activity of HDAC Inhibitors *In Vitro*

The hydroxamic acid-based HPCs (e.g., *m*-carboxy-cinnamic acid bishydroxamic acid [CBHA], suberic bishydroxamic acid [SBHA], SAHA, and pyroxamide) (Fig. 4) inhibit partially purified HDAC1 and HDAC3 at concentrations of 0.01–1.0 μ*M* *(34)*. Furthermore, the optimal concentrations of various HPCs that induce murine erythroleukemia (MEL) cell differentiation as assayed by the proportion of cells that become benzidine positive (a stain for heme of hemoglobin) are correlated directly with the concentration required to inhibit the activity of partially purified HDAC1 or HDAC3 over a wide concentration range.

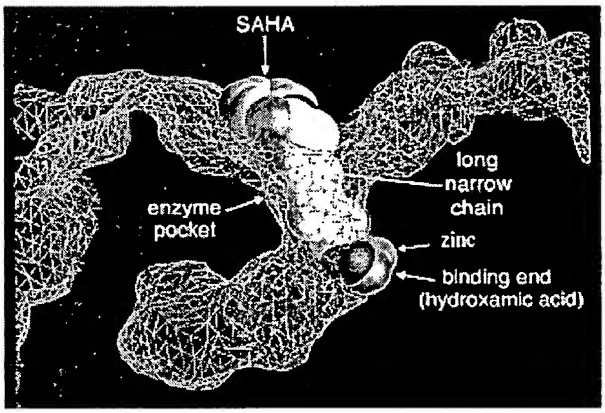


Fig. 5. SAHA (suberoylanilide hydroxamic acid) binds to the pocket of the catalytic site of a histone deacetylase-like protein, schematically represented by the netting. SAHA makes contact with residues at the rim, walls, and bottom of the pocket (enzyme pocket). The hydroxamic acid moiety of SAHA binds to the zinc at the bottom of the pocket *(80)*. (The figure is courtesy of Michael S. Finnin and Nikola P. Pavletich.)

With the use of MEL cells and T24 human bladder carcinoma cells in culture, the effects of SAHA and related hydroxamic acid-based HPCs on the acetylation of histones have been examined *(34)*. SAHA, pyroxamide, SBHA, and CBHA (Fig. 4) cause accumulation of acetylated histones. Acetylated histone type-specific antibodies were used to show that, when cells were cultured with hydroxamic acid-based HPCs, the level of acetylation in histones H2A, H2B, H3, and H4 increased (Fig. 6). Increased histone acetylation could be detected as early as 1 hour after MEL or T24 cells were cultured with SAHA or other hydroxamic acid-based HPCs. The level of acetylated histones reached a maximum 6–12 hours after the addition of HPCs and remained elevated as long as the HPC was present *(34)*.

HDAC inhibitors can induce growth arrest, differentiation, and/or apoptotic cell death in a wide variety of cultured transformed cells, including neuroblastoma, melanoma, and leukemia cells, as well as cells from breast, prostate, lung, ovary, and colon cancers *(25–30,36,40,78,81)*. For example, SAHA induces terminal cell differentiation in several cell lines, including MEL, T24 human bladder carcinoma, and MCF-7 human breast adenocarcinoma. Differentiation was evaluated by parameters that included morphology, arrest in G_1 phase of the cell cycle, and developmental markers, such as hemoglobin in MEL cells, milk proteins in MCF-7 cells, and gelsolin in T24 cells. SAHA induces apoptotic death of human multiple myeloma cells (ARP-1), human prostate cell lines (LNCaP), and myelomonocytic leukemia cells (U937). CBHA induced apoptotic cell death of several human neuroblastoma cell lines, LAI-55n, KCN-69n, and SK-N-ER. Apoptosis was assayed by DNA fragmentation analysis and the deletion of a sub-G_1 (<2N ploidy) population by flow cytometry.

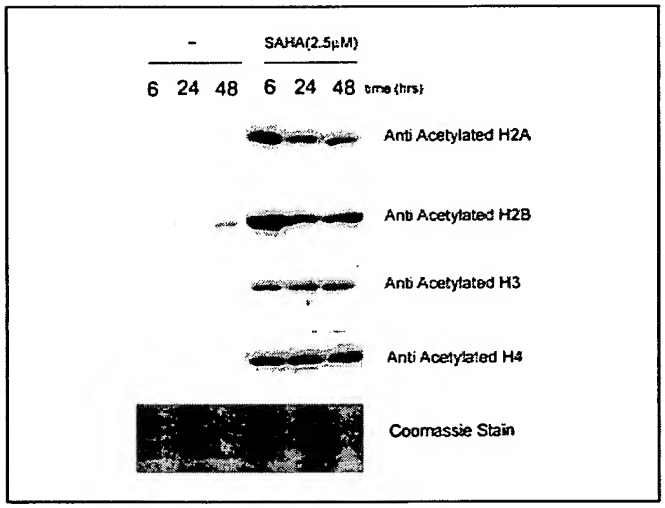


Fig. 6. Effect of SAHA (suberoylanilide hydroxamic acid) on histone acetylation in MEL cells. Cells were cultured without (−) or with 2.5 μ*M* SAHA for the times indicated. The acetylation of the histones was analyzed by use of antibodies specific for acetylated H2A, H2B, H3, and H4. The Coomassie-stained gel, **at the bottom,** indicates that the amount of protein loaded in each lane was similar [for details of methods, *see (36)*].

Van Lint et al. *(82)* have shown that the action of HDAC inhibitors on gene expression is selective. In cells cultured with TSA, the expression of only about 2% of expressed genes is changed (increased or decreased) twofold or more compared with untreated control cells. Our laboratory has obtained comparable results with transformed cells cultured with SAHA. The basis for the gene selectivity of SAHA or TSA is not known.

One gene most consistently induced by HDAC inhibitors is the cyclin-dependent kinase inhibitor $p21^{WAF1}$, which plays an important, if not determinant, role in the arrest of cell growth. Butyrate, TSA, depsipeptide, oxamflatin, MS-27-275, and the hydroxamic acid-based HPCs *(28,31,32,34,83)* induce $p21^{WAF1}$ transcription. The relation between SAHA-mediated histone hyperacetylation and increased $p21^{WAF1}$ gene expression has been studied in T24 human bladder carcinoma cells *(84)*. Increased transcription of the $p21^{WAF1}$ gene is associated with an increased level of acetylation on histones associated with the $p21^{WAF1}$ gene.

In Vivo Studies With HDAC Inhibitors

The butyrate analogue phenylbutyrate gave mixed results when tested as an HDAC inhibitor in animals and in a patient with APL. It was ineffective to moderately effective in inhibiting growth of solid tumors or leukemias, and that activity was observed only at relatively high doses *(28)*. A 13-year-old girl with relapsed APM who no longer responded to treatment with retinoic acid alone was treated with retinoic acid plus phenylbutyrate and had a complete clinical remission that was sustained for 7 months, during five treatment courses, before relapsing and becoming resistant to this treatment *(74)*. The acetylation of histones in her mononuclear blood cells was elevated during the period of administration of the phenylbutyrate. No remissions were induced in four other patients with APL *(75)*.

Several other HDAC inhibitors, including depsipeptide *(32)*, oxamflatin *(35)*, MS-27-275 *(32)*, and the hydroxamic acid-based HPCs *(37–39)*, inhibit tumor growth in animal models (Figs. 3 and 4). TSA did not inhibit the growth of a human melanoma xenograft in nude mice, but azeloic bishydroxamate did *(40)*. Treatment with HDAC inhibitors can increase the accumulation of acetylated histone in tumor tissue and/or normal tissues (e.g., spleen, bone marrow cells, and peripheral mononuclear cells). Thus, the level of acetylated histones is a useful intermediary marker of HDAC inhibitor activity.

Hydroxamic acid-based HPCs (Fig. 4) have been tested extensively in animal studies. One study *(37)* used rats with *N*-methylnitrosourea-induced mammary carcinomas. When these rats were fed SAHA (900 parts/million, continuously, beginning 7 days before the administration of *N*-methylnitrosourea), the incidence of mammary tumors was reduced by 40%, and the mean tumor volume was reduced by 78%—without side effects. Another study *(39)* used mice in which the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone induces lung tumors. When these mice were fed SAHA (900 parts/million, continuously, beginning 7 days before administration of the carcinogen to the end of the studies), the formation of lung tumors was substantially inhibited—also without toxic effects. A third rodent study *(38)* used nude mice bearing transplanted CWR22 androgen-dependent human prostate tumors. When these mice were given SAHA (25, 50, or 100 mg/kg per day) daily by intraperitoneal injection for 3 weeks, starting as soon as palpable tumors were detected, SAHA suppressed tumor growth at all three doses. With doses of 50 and 100 mg/kg per day, the mean tumor volume was reduced by 97%. Acetylation of histones H3 and H4 increased in the CWR22 tumor cells within 6 hours after SAHA was injected. Pyroxamide had similar effects on CWR22 tumor growth and the accumulation of acetylated histones (Fig. 4). When SAHA or pyroxamide was given at doses that markedly inhibited tumor growth, no toxicity, as evaluated by weight gain and histologic examination of multiple tissues at necropsy, was detected.

Conclusions

The studies summarized in this review indicate that the hydroxamic acid-based HPCs, in particular, SAHA and pyroxamide—are potent inhibitors of HDAC *in vitro* and *in vivo* and induce growth arrest, differentiation, or apoptotic cell death of transformed cells. We suggest that inhibition of HDAC activity leads to relaxation of the structure of chromatin associated with a specific set of programmed genes. The relaxed chromatin structure allows these genes to be expressed, which, in turn, arrests tumor cell growth. SAHA and pyroxamide are lead compounds among the family of hydroxamic acid-based HPCs and are currently in phase I clinical trials.

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NOTES

Supported in part by Public Health Service grant CA08748 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by grants from the Japan Foundation for the Promotion of Cancer Research and the DeWitt Wallace Fund for the Memorial Sloan-Kettering Cancer Center.

Manuscript received November 26, 1999; revised May 15, 2000; May 30, 2000.

Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms

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ABSTRACT

Purpose: **The primary objectives of this trial were to define the maximum tolerated dose (MTD) and to characterize the toxicities and pharmacokinetics of depsipeptide (FR901228) given on a day-1 and day-5 schedule every 21 days. A secondary objective of the trial was to seek evidence of antineoplastic activity.**

Patients and Methods: **Patients with advanced or refractory neoplasms received depsipeptide by a 4-h i.v. infusion on days 1 and 5 of a 21-day cycle. On the basis of preclinical data suggesting that depsipeptide may have significant cardiac toxicity, patients were treated while receiving continuous cardiac monitoring and were followed with serial cardiac enzyme determinations, electrocardiograms (ECGs), and nuclear ventriculograms (MUGA scans). The starting dose of the trial was 1 mg/m^2, and dose escalations proceeded through a total of eight dose levels to a maximum of 24.9 mg/m^2. Toxicities were graded using the National Cancer Institute common toxicity criteria, and pharmacokinetics were determined using a liquid chromatography/tandem mass spectrometry method.**

Results: **Patients (37) received a total of 88 cycles of treatment on study (range: one to eight cycles). Dose-limiting toxicity (DLT) was observed, and the MTD exceeded at a dose of 24.9 mg/m^2. The DLTs included grade-3 fatigue (3 patients), grade-3 nausea and vomiting (1 patient), grade-4 thrombocytopenia (2 patients), and grade-4 cardiac arrhythmia (1 patient, atrial fibrillation). The MTD was defined at the seventh dose level (17.8 mg/m^2). Reversible ST/T changes and mild reversible dysrhythmias were observed on the post-treatment ECG. There were no clinically significant changes in left ventricular ejection fraction. One patient achieved a partial response. The plasma disposition of depsipeptide was well described by a first-order, two-compartment model. The mean volume of distribution, clearance, $t_{1/2\alpha}$ and $t_{1/2\beta}$ at a dose of 17.8 mg/m^2 was: 8.6 liters/m^2, 11.6 liters/h/m^2, 0.42 h, and 8.1 h, respectively. The mean maximum plasma concentration at the MTD was 472.6 ng/ml (range: 249–577.8 ng/ml). Biological assays showed that the serum levels achieved could cause the characteristic cell cycle effects of this agent when serum was added to PC3 cells in culture, as well as increased histone acetylation in patient-derived peripheral blood mononuclear cells.**

Conclusion: **The MTD of depsipeptide given on a day-1 and -5 schedule every 21 days is 17.8 mg/m^2. The DLTs are fatigue, nausea, vomiting, and transient thrombocytopenia and neutropenia. Whereas cardiac toxicity was anticipated based on preclinical data, there was no evidence of myocardial damage. However, reversible ECG changes with ST/T wave flattening were regularly observed. Biologically active serum concentrations were achieved, and 1 patient obtained a partial response. The recommended Phase II dose is 17.8 mg/m^2 administered on day 1 and 5 of a 21-day cycle.**

INTRODUCTION

Depsipeptide (FR901228, NSC 630176) is a novel anticancer agent isolated from the fermentation broth of *Chromobacterium violaceum* (1). It has demonstrated potent cytotoxic activity against human tumor cell lines and *in vivo* efficacy against both human tumor xenografts and murine tumors (2, 3). Although it was identified as a Pgp[2] substrate by COMPARE analysis of its NCI drug screen cytotoxicity profile, and confirmed as such in the laboratory, depsipeptide is highly potent against sensitive cell lines in the screen (4). Depsipeptide shows a lack of cross-resistance with the cytotoxic agents vincristine, 5-fluorouracil, mitomycin C, and cyclophosphamide. Although

Received 7/26/01; revised 11/27/01; accepted 12/13/01.
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[2] The abbreviations used are: Pgp, P-glycoprotein; NCI, National Cancer Institute; MTD, maximum tolerated dose; ECOG, Eastern Cooperative Oncology Group; MUGA, ; CPK, creatine phosphokinase; LDH, lactate dehydrogenase; ECG, electrocardiogram; AUC, area under the concentration *versus* time curve; DLT, dose-limiting toxicity; HDAC, histone deacetylase; IL, interleukin; MUGA, multiple gated acquisition; AGC, absolute granulocyte count.

initially identified by Fujisawa Pharmaceutical Co. in a screening program for agents able to reverse the malignant phenotype of *Ha-ras*-transformed NIH 3T3 cells, depsipeptide has been identified recently as a potent HDAC inhibitor (3, 5). Histone deacetylation is an important component of transcriptional control, and thus, inhibition of the deacetylase enzyme may be a mechanism of antineoplastic activity of depsipeptide (5, 6). Depsipeptide, however, is structurally distinct from other known HDAC inhibitors, such as the trichostatins and trapoxins, and may have other mechanisms of cytotoxic action (7).

Laboratory studies have demonstrated that depsipeptide, like other HDAC inhibitors, induces expression of a specific subset of genes linked to inhibition of cell growth and induction of differentiation (8, 9). Depsipeptide is able to cause both a p21-dependent G_1 and a p21-independent G_2 arrest, with the G_2 arrest appearing more cytotoxic than the G_1 arrest (10, 11). In human breast cancer cells, increased p21, phosphorylation of Bcl2, and apoptosis have been observed after depsipeptide treatment (12). In thyroid cancer cells, low concentrations of depsipeptide have been shown to increase expression of a functional Na^+/I^- symporter in poorly differentiated thyroid carcinoma cells (13), thus offering a potential therapeutic strategy for resensitizing radioresistant thyroid cancer to radioiodine.

In preclinical studies, greater antitumor activity was observed with an intermittent schedule of depsipeptide administration than with daily administration because of greater host tolerance for depsipeptide and the ability to administer higher individual doses. In addition, it was observed that short infusions (>30 s to 4 min) and prolonged infusions (>24 h) caused the greatest toxicity and that infusions of 1–4 h produced the least toxicity and allowed for the highest individual doses. Two potentially serious toxicities were observed in the preclinical assessment of depsipeptide. Cardiac toxicity, including elevations in cardiac enzymes and necrosis, with chronic inflammation or neutrophilic infiltration of cardiac muscle on histopathological examination was observed in some dosing schedules. In addition, local inflammation and necrosis were noted at catheter insertion sites. This study was designed to determine the MTD and toxicity profile and characterize the pharmacokinetic profile of a 4-h infusion of depsipeptide given intermittently on a day-1 and day-5 schedule every 21 days. Precautions for potential cardiac and catheter site toxicities were included.

PATIENTS AND METHODS

The study was approved by the NCI Institutional Review Board and the Institutional Review Board of Ohio State University. All patients were required to give written informed consent before study participation.

Eligibility Criteria. Patients with histologically confirmed evidence of malignancy for whom no known standard therapy was available that was either curative or definitely capable of extending life expectancy were eligible. Other eligibility criteria included age ≥18 years, ECOG performance status ≤2, and estimated life expectancy >12 weeks. Previous chemotherapy or biological therapy had to be discontinued for at least 4 weeks. Previous radiotherapy had to be completed 3 weeks before study entry and could not have encompassed >25% of the bone marrow. Patients were required to have the following laboratory values, obtained within 14 days of study participation: neutrophils ≥1,000/μl, platelets ≥100,000/μl, creatinine ≤1.5 × upper normal limit, bilirubin ≤1.5 × upper normal limit, aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤3 × upper normal limit, prothrombin time/partial thromboplastin time ≤1.1 × upper normal limit. Patients were not eligible if they had an uncontrolled infection, central nervous system metastasis, or recent major surgery (within 21 days). Pregnant or lactating patients were not allowed on study.

Toxicity and Response Evaluation. Pretreatment evaluation included a complete history and physical examination, PT/PTT, electrolytes, renal function and liver function tests, CPK, LDH, urinalysis, MUGA scan, chest X-ray, 12-lead ECG, β-human chorionic gonadotropin (if appropriate), and HIV serology. All patients were evaluated with computed tomography scans of the chest, abdomen, and pelvis. A 24-h Holter monitor was obtained on all patients before treatment as a baseline study. Patients in the first cycle were observed with continuous cardiac monitoring during the 5-day interval starting with the first infusion on day 1 until 24 h after the second infusion on day 5. During these 5 days, patients were evaluated with daily 12-lead ECGs, CPK, LDH, and troponin-I determinations, as well as routine blood counts and serum chemistries. Patients were also evaluated by echocardiography on day 6 of each cycle. Urinalysis, cardiac enzymes, blood counts, and chemistries were performed weekly between cycles, as well as before each cycle. An ECG was performed before each cycle, and a MUGA scan was performed after every second cycle. Tumor measurements were obtained after every other cycle, and responses were scored according to WHO criteria. All toxicities were graded according to the NCI Common Toxicity Criteria. DLT was defined as any grade-4 hematological toxicity and any grade-3 or -4 nonhematological toxicity, excluding alopecia. The MTD was defined as the dose below which ≥2 of 3 or ≥2 of 6 patients experienced DLT. After determination of the MTD, 3 additional patients were treated at this dose to further define toxicity.

Dose Escalation. The starting dose was 1 mg/m^2, which represented one-third of the toxic low dose in dogs from the initial preclinical studies. At least 3 new patients were to be recruited for each dose level. Three additional patients (for a total of 6) were treated at the same dose level if 1 of the first 3 exhibited DLT. Patients who experienced grade-3 nonhematological toxicity or grade-4 hematological toxicity could continue to receive depsipeptide but at the next lowest dose level and only after all toxicity had resolved to at most grade 1. The drug was discontinued if patients experienced grade-4 nonhematological toxicity. Dose escalation followed a modified Fibonacci scheme (Table 1). The dose escalation scheme was accelerated after the 1st patient was treated at the fourth dose level. This protocol amendment was based on data from a concurrent Phase I study of depsipeptide given on a weekly schedule at Georgetown University. The accelerated fourth dose level (6.5 mg/m^2) was designated 4B.

The day-5 dose was delayed to day 7 if blood counts, renal function, or liver function studies were abnormal and not within 10% of baseline values. If repeat values on day 7 were not within the 10% margin, the drug was held until the 1st day of the following cycle. The protocol was amended after the fifth dose

Table 1 Dose levels

Level	Dose (mg/m^2)
1	1.0
2	1.7
3	2.5
4	3.5
4B	6.5
5	9.1
6	12.7
7	17.8
8	24.9

level to allow treatment on day 5 if the AGC was $\geq$1,000/mm^3 and the platelet count $\geq$75,000/mm^3.

The protocol was also amended to allow intrapatient dose escalation when the sixth dose level was reached. Patients with responding or stable disease could receive the next higher dose level after 3 patients had received that dose level without DLT and had been monitored for at least 3 weeks after treatment. Three patients had their doses escalated to the next highest dose level after having stable disease with multiple cycles of treatment on study.

Drug Administration. Depsipeptide was administered on day 1 and day 5 of a 21-day cycle as a 4-h i.v. infusion through a central venous catheter that had healed for at least 7 days after placement. Depsipeptide was reconstituted with 2 ml of diluent (containing a 4:1 mixture of propylene glycol and ethanol) from 10 mg of vials of lyophilized powder provided by the Division of Cancer Treatment, Diagnosis, and Centers/NCI and then administered after dilution in 0.9% sodium chloride to a concentration of 0.04 mg/ml.

Pharmacokinetics. Blood samples were drawn in heparinized tubes before the treatment and at 1, 2, 3, 4, 4.25, 4.5, 5, 7, 10, 16, 24, and 48 h after initiation of the depsipeptide infusion. Samples were collected before infusion, as well as 4 and 7 h after the start of the infusion on day 5. Samples collected on days 1 and 5 during the first and the second cycles were centrifuged, and the plasma was stored at −80°C until analysis. Samples were analyzed using a sensitive electrospray liquid chromatography/tandem mass spectrometry assay as described by Li and Chan (14). The data were analyzed using a two-compartment open model using the computer program ADAPT II. Pharmacokinetic parameters, volume of distribution (V_c), clearance (CL), distribution half-life ($T_{1/2}\alpha$), and elimination half-life ($T_{1/2}\beta$), were obtained by using weighted least square analysis within the same program. The highest observed concentrations were used for C_{max}. In addition, the data from the first cycle were used for model-independent analysis. For noncompartmental analysis, the area under the concentration *versus* time curve ($AUC_{0\text{-}inf}$) was calculated by using the trapezoidal rule extrapolated to infinity. The following equations were used to calculate elimination half-life ($T_{1/2}$), systemic clearance (CL_{tot}), and apparent volume of distribution at steady state (V_{dss}): $T_{1/2} = 0.693/ke$, CL_{tot} = dose/AUC, $Vd_{ss} = CL_{tot} \times$ mean residence time. The difference between cycles 1 (C1) and 2 (C2) was evaluated by the ratio of C2:C1 from patients who received the same dose for both cycles.

Table 2 Study population

Characteristic	No. of Patients
Total	37
Sex	
Male	20
Female	17
Age	
Mean	53
Range	23–75
Performance status	
ECOG 0	11
ECOG 1	24
ECOG 2	2
Tumor type	
Sarcoma	1
Leiomyosarcoma	1
Colorectal	11
Renal	12
Breast	2
Non-small cell lung	3
Melanoma	5
Head and neck	1
Adenoid cystic	1

Biological Assays

Cell Cycle Assay. An *ex vivo* biological assay was performed using serum samples from patients on the study. PC3 prostate cancer cells were grown in RPMI medium supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 incubator. Exponentially growing cells at 50–70% confluence were incubated overnight with serum from patients obtained at various pharmacokinetic time points and diluted 1:1 with normal medium. The cells were then analyzed by standard methods: trypsinized, washed with PBS, and resuspended in a propidium iodide staining solution (PBS with 0.1% Triton X-100, 1 mg/ml RNase A, and 50 μg/ml propidium iodide; Ref. 11). The suspension was then passed through a nylon mesh filter and analyzed using a Becton Dickinson FACSort.

Histone Acetylation Assay. Cytospins were prepared from patient mononuclear cells isolated from whole blood by Ficoll-Hypaque gradient. The slides were fixed in 95% ethanol/5% acetic acid for 1 min at room temperature. Depsipeptide-treated (10 ng/ml overnight) PC3 cells were included as a positive control. After fixation, slides were washed twice with PBS for 15 min, blocked in 8% BSA in PBS for 1 h at room temperature, and washed 15 min in PBS before incubating overnight at 4°C with 5 μg/ml anti-α acetylated Histone H3 (Upstate Biotechnology, Lake Placid, NY) in 2% BSA in PBS. Subsequently, cells were washed twice with PBS for 5 min at room temperature and then stained with horse antirabbit FITC-conjugated secondary antibody (Vector Laboratories, Burlingame, CA). After staining with secondary antibody, slides were washed three times with PBS for 15 min and then counterstained with 4′,6-diamidino-2-phenylindole-containing antifade compound (Vector Laboratories).

RESULTS

Thirty-seven patients received a total of 88 cycles of depsipeptide at eight different dose levels. Patient characteristics are outlined in Table 2. The planned dose escalation scheme was

Table 3 Hematological toxicity in cycle 1

Grade dose mg/m²	*n*	Thrombocytopenia				Neutropenia			
		1	2	3	4	1	2	3	4
1.0	*3*								
1.7	*3*								
2.5	*3*								
3.5	*1*	1					1		
6.5	*3*	2				2			
9.1	*3*	3					1	1	
12.7	*3*	1		1		1	2		
17.8	*9*	2	2	5		3	2	4	
24.9	*8*	2	1	3	2	2	3	1	

modified in the course of the study. The following dose levels were evaluated: 1, 1.7, 2.5, 3.5, 6.5, 9.1, 12.7, 17.8, and 24.9 mg/m² on days 1 and 5 (Table 1). Only 1 patient was treated at a dose of 3.5 mg/m², because the dose escalation scheme was modified at dose-level 4 (see "Patients and Methods"). Patients (36) were assessable for toxicity during the first cycle of therapy. One patient was not assessable, because he required surgical intervention for hemoptysis after the day-1 treatment. This patient was not retreated until he recovered from surgery. At that point, his dose level had been identified as safe, and patients had been enrolled beyond 9.1 mg/m². He was, therefore, considered inevaluable for toxicity. Two additional patients did not receive the day-5 dose during the first cycle of treatment. One patient at dose-level 2 (1.7 mg/m²) developed an increase in CPK with absence of MB fraction, associated with a fever, and the second, entered at 24.9 mg/m², developed erythema around the central venous access device site. Three patients had the day-5 dose delayed in the first cycle of treatment. One was delayed because of a decreased neutrophil count (at 17.8 mg/m²), a second because of a decreased platelet count (at 9.1 mg/m²), and the third because of a central venous catheter clot (at 17.8 mg/m²). All 3 received the second dose without complications.

Two patients were removed from the study for potential toxicity. The 1st occurred at the first dose level because of a persistent elevation of CPK above normal without other findings. The MB fraction remained normal. The rise in CPK was evident on the 12th day of the second cycle (573 units/dl; normal: 52–386 units/dl) of treatment and returned to normal by the 5th day of cycle 3 (327 units/dl). The CPK rose again by the 12th day of cycle 3 (576 units/dl), and the patient was taken off study. The CPK was followed to day-70 post-treatment and fluctuated outside of the normal range (444–627 units/dl). There were no associated ECG, LDH, or troponin-I abnormalities or evidence of myopathy. No other patients experienced CPK elevations that could be related to depsipeptide treatment. The 2nd patient, enrolled at the 17.8 mg/m² dose level, was removed from the study after the development of splenic infarcts.

Hematological Toxicity. The number of patients experiencing neutropenia or thrombocytopenia during the first cycle at each dose level is outlined in Table 3. Overall, significant hematological toxicity did not occur at dose levels <9.1 mg/m² on any cycle of treatment. Two patients with grade-4 thrombocytopenia were among the cycle-1 toxicities at 24.9 mg/m² that were defined as dose limiting. Episodes of neutropenia and thrombocytopenia had a distinct time course unlike that of chemotherapeutic agents in current clinical use. As in the examples shown in Fig. 1, there was a rapid drop in both platelet count and neutrophil count, with a rapid recovery. Data from cycles 1 (○), 2 (●), and 3 (■) are shown, with no evidence of cumulative toxicity.

We also evaluated multiple treatment cycles at each dose level. At 9.1 mg/m², out of a total of eight cycles of treatment given to 3 patients, 1 patient had the day-5 dose delayed for 1 day because of thrombocytopenia (platelet count = 110,000/mm³). A 2nd patient had the day-5 dose delayed for 1 day because of neutropenia (AGC = 740/mm³, C3) and had the day-5 dose omitted in two cycles because of both neutropenia and thrombocytpenia [AGC = 934/mm³ (C2) and 785/mm³ (C4); platelet counts = 85,000/mm³ (C2) and 61,000/mm³ (C4)]. This patient was responding to treatment and, therefore, had escalation of the day-1 dose after safety was confirmed in other cohorts. This patient continued to have the day-5 dose omitted in the next four cycles, one given at 12.7 mg/m² and three at 17.8 mg/m². Excluding this patient, but including all other patients who received at least one cycle of depsipeptide at 17.8 mg/m², there were 26 treatment cycles in 13 patients at 17.8 mg/m². Among these patients, none had day-5 dose delays or omissions for thrombocytopenia, and only 2 patients had the day-5 dose omitted for neutropenia. There were a total of 13 episodes of grade-3 and 3 episodes of grade-4 neutropenia and 9 episodes of grade-3 and 2 episodes of grade-4 thrombocytopenia in the 26 cycles received by this same group of 13 patients. Whereas too few patients received multiple cycles of treatment at dose levels >9.1 mg/m² to make conclusive judgements, the transient hematological toxicity of depsipeptide does not seem cumulative.

Nonhematological Toxicity. Table 4 summarizes the major nonhematological toxicities associated with the first cycle of depsipeptide for each dose level. Drug-related grade-3 or -4 nonhematological toxicities were defined as dose limiting and were noted in the first cycle in patients receiving 24.9 mg/m². The most common toxicity was severe fatigue, observed in 3 patients in the first cycle at 24.9 mg/m². One patient had grade-3 nausea and vomiting followed by an episode of atrial fibrillation requiring treatment (grade 4) on day 7. One patient transiently developed grade-3 hypocalcemia on day 2, and 2 patients developed grade-3 hypophosphatemia on days 5 and 10, respectively. Together with the two episodes of grade-4 thrombocytopenia, these nonhematological toxicities occurring in the first cycle of therapy with depsipeptide defined 24.9 mg/m² as a dose that exceeded the MTD. Only one grade-3 nonhematological toxicity was observed in the first cycle at a dose <24.9 mg/m², and that was transient grade-3 hypocalcemia occurring on day 6 in a patient treated at 17.8 mg/m². This was not considered related to depsipeptide until it was also observed in a patient receiving 24.9 mg/m².

Nonhematological toxicities occurring in multiple cycles of therapy were similar to those observed after the first cycle. Six patients experienced grade-3 fatigue after either the first or subsequent cycles of depsipeptide, 1 patient at a dose of 17.8 mg/m² and 5 patients at a dose of 24.9 mg/m². Grade-2 fatigue was observed in 6 of 26 cycles administered to 13 patients at

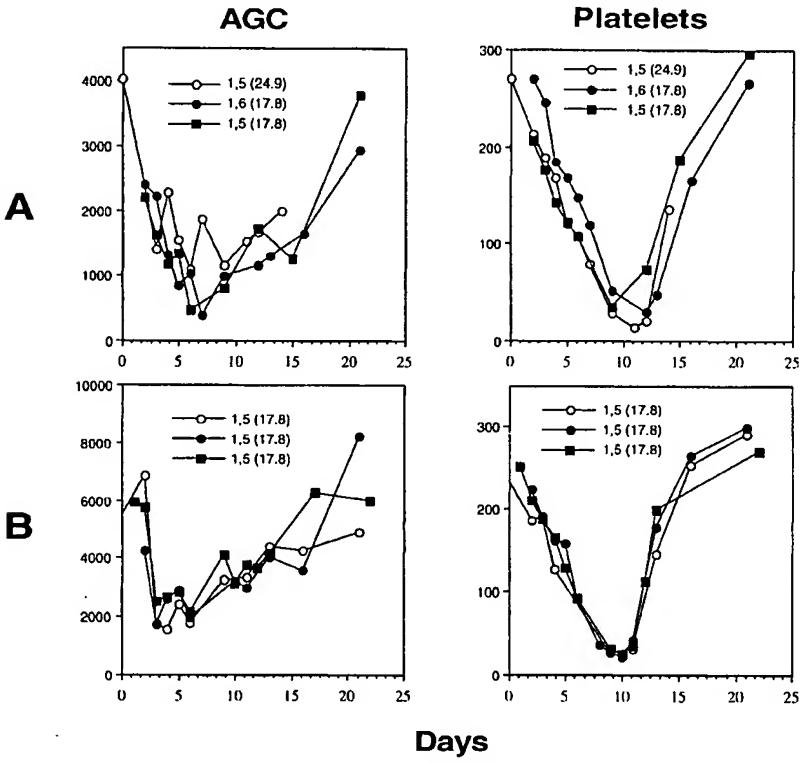

Fig. 1 Hematological effects of depsipeptide. Counts from the first three cycles in 2 patients (*A* and *B*) were plotted after treatment. Results from cycle 1 are shown in the ○, cycle 2 with the ●, and cycle 3 with the ■. These patients received 17.8 mg/m^2, except in *A*, where the patient received the first cycle of depsipeptide one dose above the MTD. The legend indicates whether the day-5 dose was administered on day 5 or day 6 and the dose received (mg/m^2).

Table 4 Nonhematological toxicity in cycle 1

	17.8 mg/m^2 (n = 9)				24.9 mg/m^2 (n = 8)			
	Patients with any toxicity		Patients with grades 3 and 4 toxicity		Patients with any toxicity		Patients with grades 3 and 4 toxicity	
Toxicity	No.	%	No.	%	No.	%	No.	%
Anorexia	4	44	0	0	5	63	2	25
Cardiac								
EKG[a] changes	7	78	0	0	7	88	0	0
Dysrhythmia	1	11	0	0	2	25	1	13
Fatigue	6	67	0	0	8	100	3	38
Fever	7	78	0	0	7	88	0	0
Nausea	7	78	0	0	8	100	1	13
Vomiting	6	67	0	0	8	100	1	13
Taste change	6	67	0	0	3	38	0	0
Headache	4	44	0	0	2	25	0	0
Hypocalcemia	7	78	1	11	5	63	1	13
Hypophosphatemia	1	11	0	0	4	50	2	25

[a] ECG, electrocardiogram.

17.8 mg/m^2. Two patients refused additional therapy because of fatigue. Initially in the study, patients were not given routine nausea and vomiting prophylaxis. At doses of ≥3.5 mg/m^2, patients began to experience grade-2 nausea and vomiting. These symptoms were often delayed, and patients were subsequently given prophylaxis with ondansetron, prochlorperazine, and occasionally metoclopromide. Grade-2 nausea and/or vomiting was noted in 12 of 26 cycles administered in 13 patients at 17.8 mg/m^2; no grade-3 episodes were noted at this dose level. Hypocalcemia occurred in 15 (grade 2) and 1 (grade 3) of 26 cycles administered in 13 patients at 17.8 mg/m^2 and in 8 (grade 2) and 1 (grade 3) of 14 cycles administered in 8 patients at 24.9 mg/m^2, but the episodes were not associated with symptoms or significant clinical findings. Alopecia was not observed. Despite concerns regarding catheter-site toxicities generated by the preclinical data, only 1 patient experienced a catheter-site complication that was not thought attributable to depsipeptide administration.

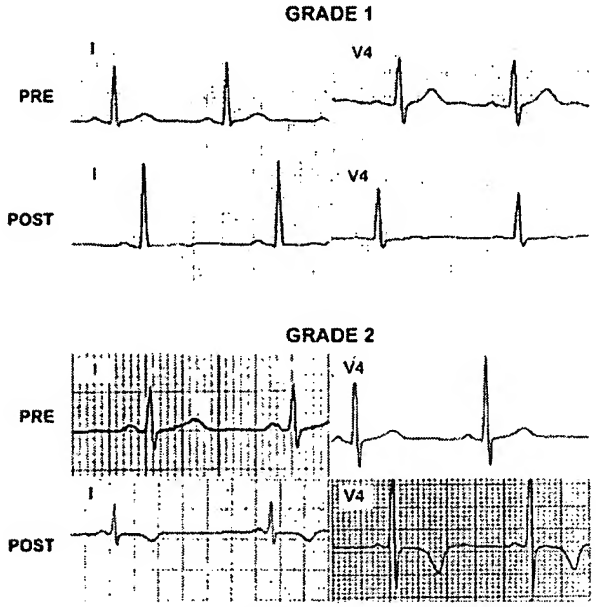

Fig. 2 ECG changes in patients receiving depsipeptide. Grade-1 changes were defined as ST/T-wave flattening, whereas ST segment depression and inversion were classified as grade 2.

Cardiac Monitoring and Toxicity. A major concern during the protocol, based on the preclinical studies, was to identify any occurrence of cardiac toxicity. Because of this, patients were monitored for cardiac toxicity as described in the "Patients and Methods" section above. Beginning with the fourth dose level (3.5 mg/m^2), ECG changes consisting of T-wave flattening in the anteriolateral or inferior leads were observed (grade 1; Fig. 2). At a dose of 24.9 mg/m^2, 2 patients developed deep T-wave inversions with ST-segment depression (grade 2; Fig. 2), which returned to normal before the subsequent cycle. The ECG changes were noted to some degree within 24 h and were most marked on day 6, the day after the second dose of the cycle. Because ECG changes were not obtained between days 6 and 21, it is not known how long the ST/T-wave changes persisted. Three patients experienced asymptomatic arrhythmias after depsipeptide, detected during the inpatient cardiac monitoring period. Episodes included asymptomatic atrial bigeminy (on a background of preexisting sinus bradycardia) in 1 patient, a 3-s sinus pause while a 2nd patient was sleeping, and an asymptomatic five beat run of ventricular tachycardia in a 3rd patient. None of these was considered a DLT or definitely related to depsipeptide. One patient, described above, experienced an episode of atrial fibrillation that was symptomatic and dose limiting. The patient was subsequently retreated at a lower dose (17.8 mg/m^2) without recurrence of the atrial fibrillation. Cardiac ejection fraction was measured in all patients before treatment and subsequently as described in "Patients and Methods." The mean pretreatment ejection fraction was 56.4 ± 9.1%, and the mean of all post-treatment ejection fraction measurements was 53.1 ± 5.7%, values not found to be significantly different, $P = 0.097$. No patient developed any degree of clinical heart failure or a measured ejection fraction outside of the normal range. Among patients receiving doses of ≥17.8 mg/m^2, the mean ejection fraction pre and postdepsipeptide was 57.3 ± 7.4% and 56.6 ± 5.4%, respectively. Finally, no Troponin-I elevations were observed.

Pharmacokinetics. Table 5 provides an overview of the pharmacokinetic parameters of depsipeptide at the various dose levels in this trial. The plasma disposition of depsipeptide was well described by a first-order, two-compartment open pharmacokinetic model. Fig. 3 shows concentration *versus* time curves generated using this model and data from 2 different patients treated at 1 (Fig. 3*A*) and 17.8 mg/m^2 (Fig. 3*B*). Using the model, the mean volume of distribution, clearance, distribution half-life, and elimination half-life with a dose of 17.8 mg/m^2 was 8.6 liters/m^2, 11.6 liters/h/m^2, 0.42 h, and 8.1 h, respectively, with two compartmental analysis. In addition, the maximum concentration (C_{max}) and AUC of depsipeptide increased as the dose increased (Fig. 4, *A* and *B*). The ratio of C_{max} and AUC for C2 *versus* C1 showed no apparent differences. Both two-compartmental and noncompartmental methods of analysis provided similar pharmacokinetic parameters (Table 5, *A* and *B*). As such, either method can be used for pharmacokinetic analysis in future studies with depsipeptide.

Biological Assays. To confirm that the level of depsipeptide in the serum was sufficient to achieve the biological effects observed *in vitro,* two different assays were evaluated. First, serum was obtained from patients before treatment with depsipeptide and then at subsequent time points. This serum was added in a 1:1 dilution with normal media to a monolayer of proliferating PC3 cells. As a control, 10 ng/ml depsipeptide were added to PC3 cells alone without any patient serum. Fig. 5 shows the results obtained with serum derived from a patient at the indicated time points after treatment with 17.8 mg/m^2 depsipeptide. In the *top right panel,* there is a cell cycle arrest after the *in vitro* addition of 10 ng/ml depsipeptide. Similarly, the addition of patient serum obtained between 4 and 5 h after initiation of the depsipeptide infusion results in a cell cycle arrest. However, with serum obtained at 7 h, the degree of cell cycle arrest is minimal. This correlates well with the elimination half-life of 8.1 h obtained from pharmacokinetic analysis. Comparable results were obtained for all patients enrolled at the MTD. As a second assay performed to assess depsipeptide effects, histone acetylation was evaluated by immunofluorescence. Normal circulating mononuclear cells were obtained from patients pre and post-treatment. As shown in Fig. 6, there is an increase in histone acetylation in PC3 cells after *in vitro* treatment with depsipeptide. In the *second* and *third rows,* representative patient mononuclear cells are shown before and after treatment with depsipeptide (4 and 24 h in the *second* and *third rows,* respectively). Increased histone acetylation is observed. These results can be compared with the increase in histone acetylation observed in normal mononuclear cells to which depsipeptide has been added *in vitro* (*bottom row*).

Responses. As this was a Phase I trial, the response was a secondary end point. One partial response was observed at a dose of 9.1 mg/m^2 in a patient with renal cell carcinoma. Eight patients had stable disease at the initial evaluation, 24 patients had progressive disease, and 4 patients were not evaluable for response. The patient with a partial response had a >50%

Table 5 Pharmacokinetic parameters of depsipeptide (FR901228) of two-compartment open model (*A*) and noncompartmental analysis (*B*)

A. Dose[a] (mg/m²)	CL[b] (liters/h/m²)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_c (liters/m²)	C_{max} (ng/ml)
1.0 (n = 3, c = 5)	8.8 ± 4.5	0.24 ± 0.14	8.3 ± 13.4	3.7 ± 2.6	35.6 ± 22.2
1.7 (n = 3, c = 5)	12.0 ± 7.5	0.61 ± 0.38	49.5 ± 58.6	9.2 ± 3.4	40.8 ± 20.9
2.5 (n = 3, c = 6)	21.6 ± 17.6	0.48 ± 0.68	16.1 ± 31.2	9.3 ± 10.1	49.5 ± 31.7
3.5 (n = 1, c = 2)	10.6 ± NA	0.63 ± NA	9.5 ± NA	10.5 ± NA	129.9 ± NA
6.5 (n = 3, c = 5)	10.5 ± 2.2	0.30 ± 0.11	6.6 ± 2.2	5.0 ± 1.6	211.8 ± 77.1
9.1 (n = 4, c = 7)[c]	21.7 ± 11.4	1.20 ± 2.3	19.0 ± 24.5	10.5 ± 13.5	162.6 ± 53.8
12.7 (n = 3, c = 5)	38.2 ± 26.4	0.26 ± 0.20	12.7 ± 9.1	20.7 ± 20.2	224.7 ± 241.0
17.8 (n = 11, c = 19)	11.6 ± 5.8	0.42 ± 0.25	8.1 ± 6.0	8.6 ± 6.3	553.8 ± 299.5
24.9 (n = 8, c = 12)	19.2 ± 10.6	0.34 ± 0.18	4.3 ± 2.3	8.6 ± 3.3	478.2 ± 316.6

B. Dose[c] (mg/m²)	n	AUC_{0-t} (μg/ml/h)	AUC_{0-inf} (μg/ml/h)	$T_{1/2}$ (h)	Vd_{ss} (liters/m²)	CL_{tot} (liters/h/m²)
1.0	3	0.13 ± 0.09	0.14 ± 0.10	11.2 ± 14.9	48.8 ± 65.5	10.2 ± 6.9
1.7	3	0.15 ± 0.07	0.16 ± 0.07	14.4 ± 9.8	38.5 ± 27.0	21.5 ± 9.7
2.5	3	0.12 ± 0.11	0.12 ± 0.11	2.3 ± 2.9	38.5 ± 27.0	35.4 ± 26.5
3.5	1	0.23 ± NA	0.24 ± NA	11.8 ± NA	37.9 ± NA	14.7 ± NA
6.5	3	0.52 ± 0.06	0.53 ± 0.06	8.1 ± 3.7	18.8 ± 3.7	12.5 ± 1.4
9.1	4	0.75 ± 0.50	0.79 ± 0.49	20.6 ± 16.3	126.8 ± 155.0	14.5 ± 7.4
12.7[d]	1	0.35 ± NA	0.35 ± NA	8.5 ± NA	55.3 ± NA	34.1 ± NA
17.8	9	2.21 ± 1.35	2.27 ± 1.34	11.7 ± 8.7	37.1 ± 43.8	10.5 ± 6.4
24.9	8	1.94 ± 1.50	1.95 ± 1.52	7.5 ± 5.5	20.4 ± 8.3	18.5 ± 10.1

[a] Values, mean ± SD from cycles 1 and 2.

[b] CL, systemic clearance; $T_{1/2\alpha}$, distribution half-life; $T_{1/2\beta}$, elimination half-life; V_c, volume of distribution of central compartment; C_{max}, observed maximum concentration; n, number of patients per dose group (patients who had a dose reduction with the second cycle; the data is reflected for both cycles); c, number of pharmacokinetic profiles per dose group; V_{ss}, volume of distribution at steady state; AUC_{0-t}, AUC time curve (0 to the last measurable concentration time point); AUC_{0-inf}, AUC time curve (0 to infinity).

[c] One patient from the dose group was excluded from the toxicity evaluation; Values, mean ± SD from cycle 1.

[d] Pharmacokinetic profile was not available from 2 patients in the dose group.

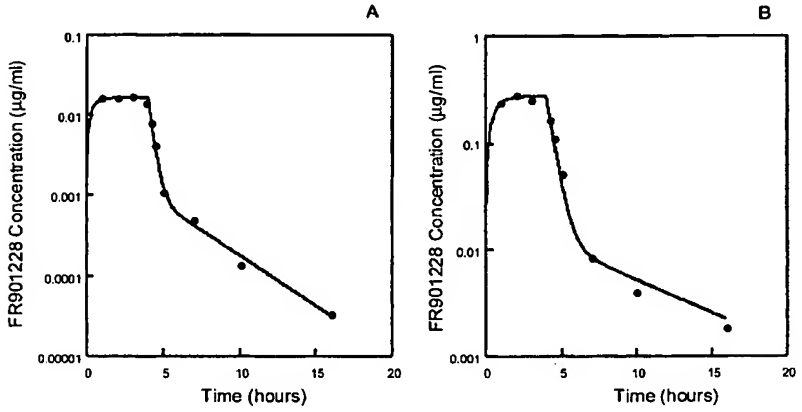

Fig. 3 Depsipeptide plasma concentration time curves from 2 representative patients receiving different dosing regimens. *A*, 1 mg/m²; *B*, 17.5 mg/m²; O, observed concentrations; ———, fitted for two-compartment model.

reduction in size of supraclavicular, mediastinal, and retroperitoneal adenopathy. This response was observed after two cycles and persisted for an additional six cycles.

DISCUSSION

This Phase I study of depsipeptide was conducted to determine the MTD and define the toxicity of this novel agent when given as a 4-h infusion on a day-1 and day-5 schedule of a 21-day cycle. DLTs were fatigue, nausea, vomiting, and thrombocytopenia. One patient developed atrial fibrillation during an episode of severe nausea and vomiting. The MTD was defined as 17.8 mg/m² given over 4 h. Biological assays confirmed the ability of serum from patients treated with depsipeptide to induce cell cycle arrest in PC3 cells and confirmed an increase in histone acetylation in circulating mononuclear cells.

It has been demonstrated that FR901228 is a HDAC inhibitor (5). Histone acetylation provides an enzymatic mechanism to regulate transcription by affecting the interaction between DNA and histones. HDAC inhibitors have been shown to induce expression of genes linked to growth inhibition and cellular differentiation (15, 16). HDAC inhibitors synergize with retinoic acid to stimulate leukemia cell differentiation (17–19). We observed induction of the multidrug-resistance gene MDR1/Pgp and epidermal growth factor with features of a differentiated

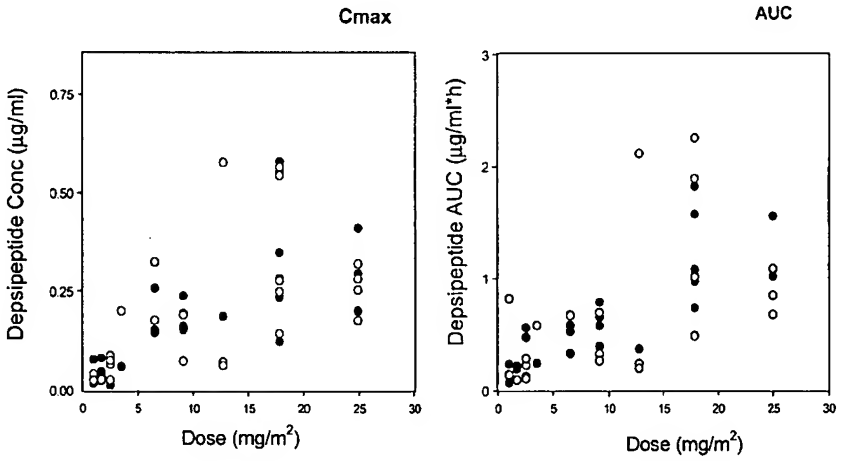

Fig. 4 C_{max} (observed maximum concentration) and AUC (area under the concentration *versus* time curve) as a function of administered dose. ●, cycle 1; ○, cycle 2.

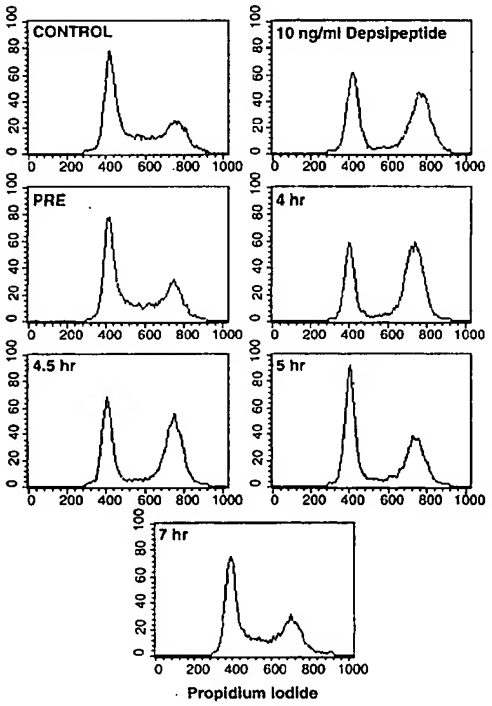

Fig. 5 *Ex vivo* assay for depsipeptide in patient serum. PC3 cells were plated in six-well dishes overnight and then treated for 24 h with drug-free media ("PC3 control" histogram), media containing 10 ng/ml depsipeptide (control + 10 ng/ml depsipeptide histogram), or media diluted 1:1 with patient serum. Serum was collected preinfusion and at various time points after the start of infusion (Pre, 4-h, 4.5-h, 5-h, and 7-h histograms). Note the lack of depsipeptide effect at 7 h after the start of infusion.

phenotype in human colon cancer and neuroblastoma cell lines after the addition of the HDAC inhibitor sodium butyrate (20–22). Likewise, depsipeptide induces multidrug-resistance gene 1 expression in human colon cancer cell lines (data not shown). Unlike sodium butyrate, which has also been studied in clinical trials, depsipeptide is active in the nM range, and the induced Pgp is functional and able to transport rhodamine.

Although both neutropenia and thrombocytopenia occurred after administration of depsipeptide, the drop in cell counts was rapid and transient, suggesting a mechanism other than toxicity to early myeloid precursors as occurs with myelosuppresive cytotoxic agents in current clinical use. Bone marrow aspirates and biopsies were not performed as a part of this study; therefore, the level of maturation at which the myelosuppresive action of depsipeptide occurs remains unknown. Although patients had delays in the administration of the day-5 dose because of either thrombocytopenia, neutropenia, or both, no complications referable to those toxicities were observed. Taking into account that the thrombocytopenia and neutropenia were maximal at 10 and 5 days, respectively, similar to the life span in blood, these results suggest that depsipeptide may affect mature cells.

On the basis of preclinical data, both cardiac toxicity and catheter site complications were expected to be dose limiting. To maximize safety in the conduct of the trial, cardiac monitoring on this study was extensive, and central venous access catheters were allowed to heal for 7 days before dosing. There was no evidence of skin necrosis at the catheter site, and skin injury sites, such as needle sticks for pharmacokinetic sampling, healed normally without excessive bleeding or evidence of skin necrosis, a discrepancy between the preclinical data and the results of the trial.

Cardiac toxicities recognized in this trial consisted mainly of asymptomatic dysrhythmias and nonspecific ST/T changes on the 12-lead ECG. With continuous cardiac monitoring, three episodes of asymptomatic dysrhythmias and one episode of symptomatic atrial fibrillation requiring treatment were recorded. We cannot ascertain whether these episodes represent cardiac toxicity because of depsipeptide or dysrhythmias that would have occurred nevertheless but were detected because of the intensive monitoring. Serial measurements of cardiac output by MUGA scanning showed no clinically significant decline in cardiac output before and after treatment. The possibility that a greater degree of cardiac toxicity could become manifest with a more prolonged treatment cannot be excluded, and cardiac mon-

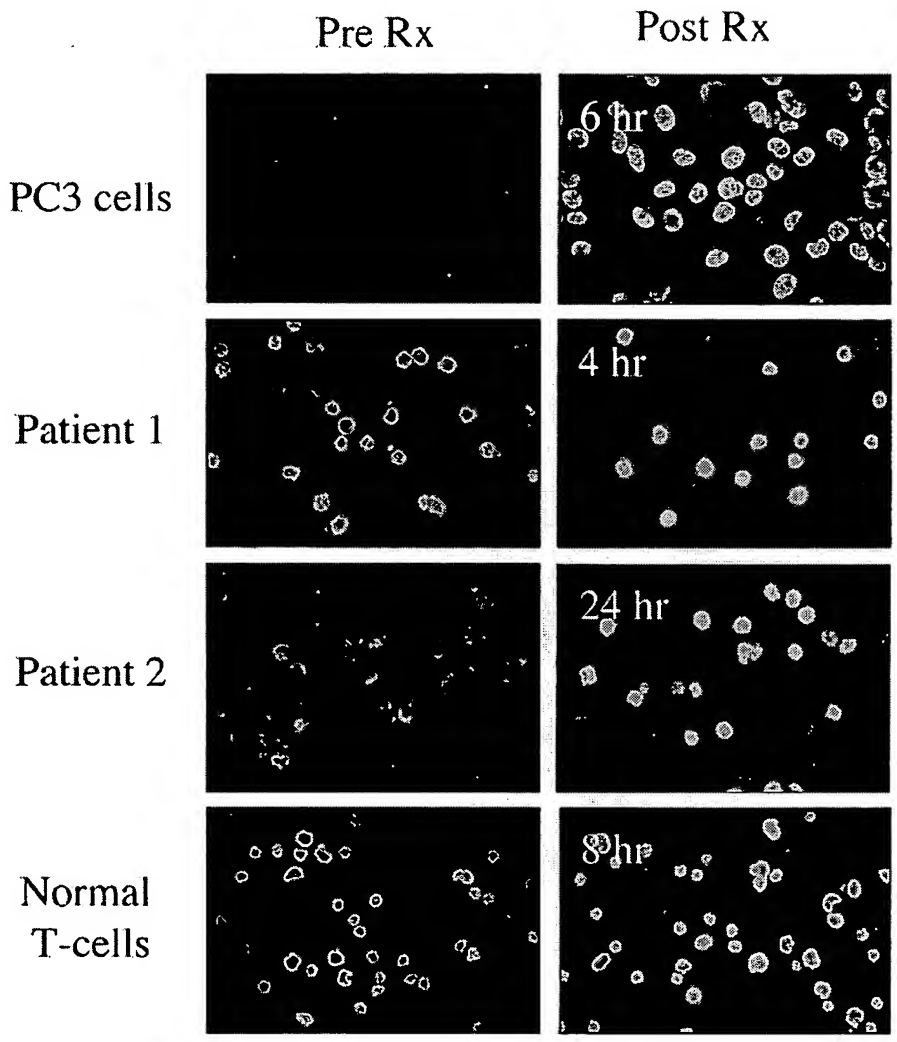

Fig. 6 Acetylated histone H3 in normal peripheral mononuclear cells. Untreated PC3 cells and PC3 cells treated for 6 h with 10 ng/ml depsipeptide are shown in the *top row*; mononuclear cells obtained from 2 patients preinfusion and 4 and 24 h after treatment with 17.8 mg/m^2 are shown in the *second* and *third rows*, respectively. Mononuclear cells obtained from a normal volunteer that were incubated in media alone or in media containing 10 ng/ml depsipeptide for 8 h are shown in the *bottom row* of *panels*. Cells were stained with an antiacetylated histone H3 antibody. Increased staining is seen in all cells post-treatment with depsipeptide.

itoring should be included in additional trials of this agent. Nausea and vomiting were associated with depsipeptide infusion. The nausea was delayed and, at the level which exceeded the MTD, was only partially responsive to ondansetron, prochlorperazine, and metoclopromide. It is possible that some of the electrocardiographic abnormalities observed were related to the use of these antiemetics, which have been reported to precipitate electrocardiographic changes and arrhythmias (23–27).

The major DLT of depsipeptide was profound fatigue. At 24.9 mg/m^2, all patients experienced some degree of fatigue, and one-third of patients experienced fatigue that was profound, functionally limiting, and lasted up to 1 week. The fatigue was not associated with anemia and was not clearly associated with other defined biochemical abnormalities. The 4 patients experiencing hypophosphatemia at 24.9 mg/m^2 may indicate that depsipeptide could induce cellular changes in ATP levels or availability. At the MTD, only 1 patient experienced dose-limiting, grade-3 fatigue in cycle 3, which recurred in cycle 4, although his dose was reduced to 12.7 mg/m^2. Six of the 9 patients enrolled at the MTD experienced some fatigue in the first cycle, and in the total 26 cycles administered to 13 patients at 17.8 mg/m^2, regardless of dose level of entry, there were 15 episodes of fatigue.

In summary, the toxicities attributable to depsipeptide administered in a day-1 and day-5 schedule are distinct from those associated with conventional chemotherapeutic agents. There was no alopecia observed, no mucositis, and a rapid recovery of blood counts that was dissimilar to the bone marrow suppression, resulting from an anthracycline or an alkylating agent. The chief DLT was fatigue; this was mild at the MTD but severe at doses exceeding the MTD. Interestingly, grade-3 toxicities for another HDAC inhibitor, tributyrin, consisted of nausea, vomiting, and myalgia, whereas fatigue occurred as a grade-1 toxicity (28). In a trial combining IL-2 with arginine butyrate, fatigue was the DLT (29). Although fatigue as a toxicity may also be nonspecific, it is tempting to speculate that it is related to HDAC inhibition.

HDAC inhibitors like depsipeptide have great potential as

anticancer agents, because the compounds induce expression of genes involved in cellular differentiation. This constellation of genes includes several that make attractive targets for combined anticancer therapy. We have shown in laboratory studies that the sodium-iodide symporter is induced in thyroid cancer cells (13). This would make depsipeptide potentially useful in combination with radioiodine. The HDAC inhibitor arginine butyrate has been shown in preliminary studies to induce expression of the IL-2 receptor (30). This receptor is the therapeutic target for an IL-2 toxin conjugate that has been approved for treatment of patients with cutaneous T-cell lymphoma (31). Indeed, preliminary results using depsipeptide in patients with cutaneous T-cell lymphoma suggests significant activity in that disease (32). Finally, there are *in vitro* studies suggesting that combination therapy with retinoids and nonretinoid differentiating agents may be more effective than retinoids alone (33, 34). If depsipeptide was to induce expression of the retinoic acid receptor, a basis for combined therapy with retinoids would be established. On the basis of its potency and cytotoxicity, depsipeptide appears to be a novel and potent HDAC inhibitor that may allow this class of drugs to enter clinical use.

ACKNOWLEDGMENTS

We thank the contributions of members of Fujisawa Pharmaceutical Co. for the supply of the compound and helpful review of the data and Ed Sausville and the Developmental Therapeutics Program for shepherding FR901228 through preclinical development. We also thank Wilma Medina-Pérez and Dr. Susan Mertins for cell culture work.

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